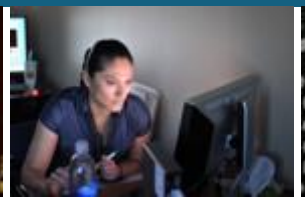
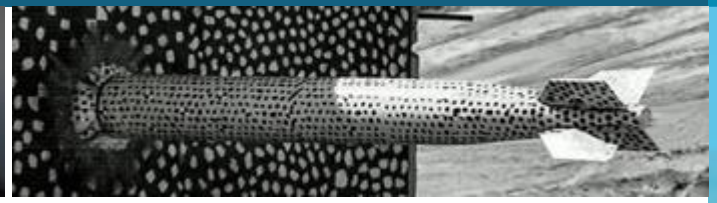
Biosensors and Diagnostics for Pandemic Response





PRESENTED BY

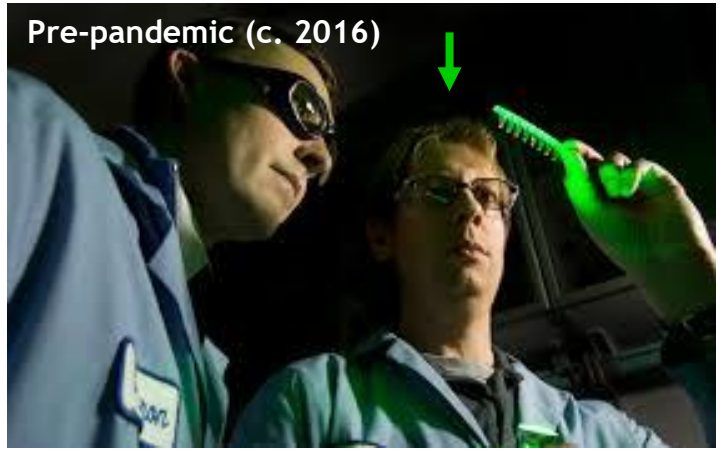
Robert Meagher – Sandia National Laboratories

Georgia Institute of Technology · ILLINOIS · PURDUE UNIVERSITY · THE UNIVERSITY OF NEW MEXICO · THE UNIVERSITY OF TEXAS AT AUSTIN

SAND2020-12307 PE

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525.

About myself and Sandia Biosciences



Robert Meagher, Ph.D.

- Principal Member of Technical Staff
- 15 years at Sandia, starting as a postdoc
- Research interests: Microfluidics, nucleic acid amplification, virus detection

Sandia Biosciences

- Interdisciplinary research groups located both in Livermore, CA and Albuquerque, NM
- Broad mission areas are **Biodefense and Emerging Infectious Disease** and **Bioenergy**
- Traditionally has leveraged Sandia capabilities in device & systems engineering for applications in **Biosensors**, **Diagnostics**, and **Detection**, including early foundational work in microfluidics
- The Biosciences groups collaborates broadly with other programs in Sandia, including programs in systems analysis, homeland security, global biosecurity, and other areas

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Sandia Diagnostics for COVID-19 Response

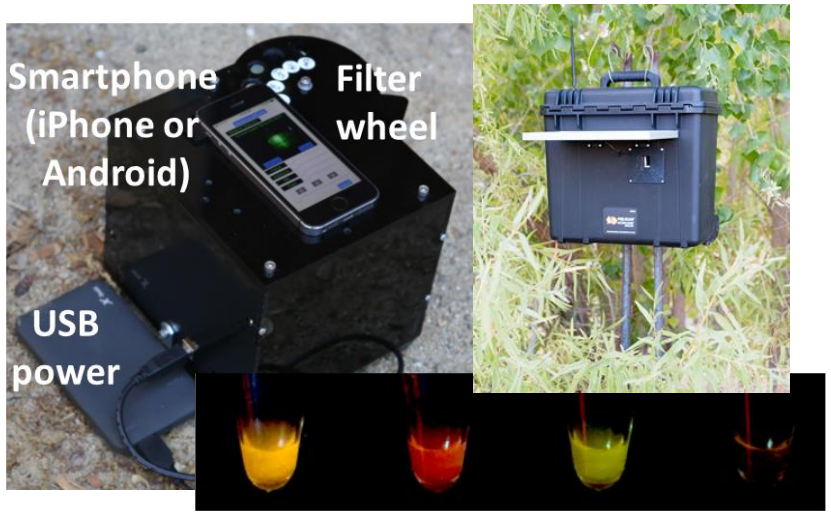
- Starting in March, Sandia as well as DOE Office of Science saw an urgent need to respond to the growing COVID-19 pandemic in the USA.
- Numerous efforts within Sandia and across the DOE Labs were rapidly initiated, leveraging multiple capabilities within the national labs (not just diagnostics).
- Two specific technologies that I have been involved with:
  - Viral RNA detection – QUASR / Smart LAMP
  - Viral protein / antibody detection - SpinDx

https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing.html

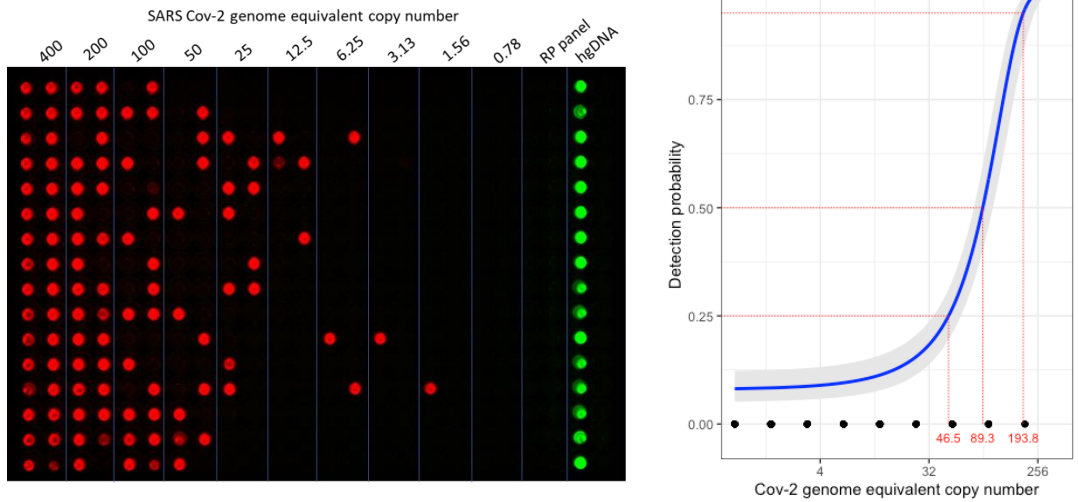
"QUASR" and Smart LAMP – Rapid Viral RNA detection

- From previous research (LDRD, DTRA, and NIH funding), we had been working with isothermal diagnostics (LAMP), especially focused on arbovirus detection, and worked to advance:
  - "Direct" amplification from viruses (circumventing extraction)
  - "QUASR" detection chemistry that enabled much higher specificity as well as very bright signals that could be discerned with simple instrumentation, *e.g.* smartphone.
- We pivoted to SARS-CoV-2 detection, and are currently seeking commercialization opportunities.
- But we're also interested in advancing the basic science of isothermal amplifications.

Smart Trap, Smart LAMP, QUASR for arboviruses (2013-2017)



SARS-CoV-2 Detection & Diagnostics (saliva, swabs, environmental)



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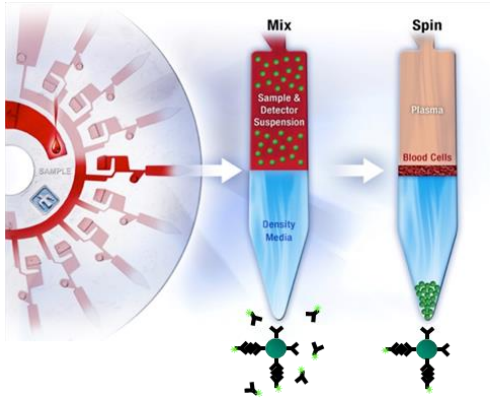
SpinDx – Portable instrument for multiplexed immunoassays

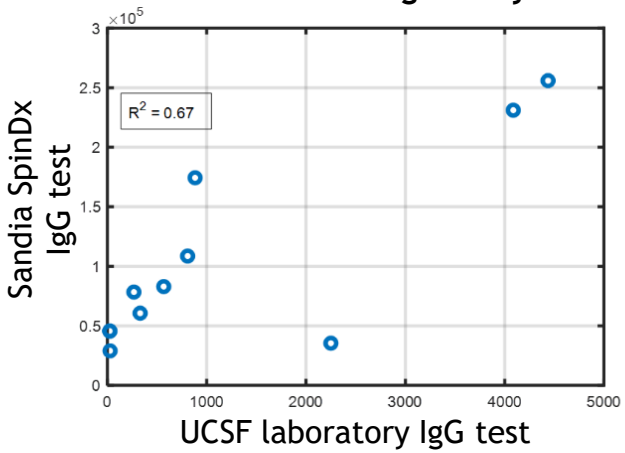
- Sandia began developing “SpinDx”, a portable bead-based sedimentation immunoassay, around 2009
  - Numerous applications in biodefense, emerging infectious disease, bioenergy
  - Mostly direct antigen detection, but also serology and nucleic acid detection
- SARS-CoV-2 pandemic has brought serology “test strips” into commom use
  - Challenges include high variability from manufactuer to manufacturer, and unclear significance of antibody results
- Sandia SpinDx enables quantitative, multi-analyte immunoassays and even parallel immunoassay + nucleic acid assay

SpinDx prototype reader

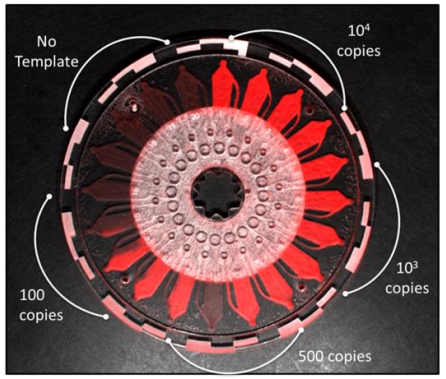
Bead-based assay principle



SARS-CoV-2 IgG Assay



Ebola virus RNA assay



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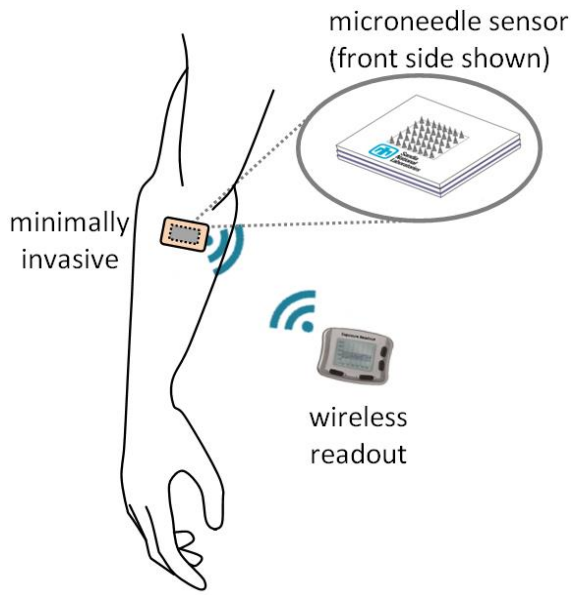
Other Sandia Biosensing/Diagnostic technologies (Slide 1 of 2)



Wearable Micro-Needle Array based sensor for bioagent exposure

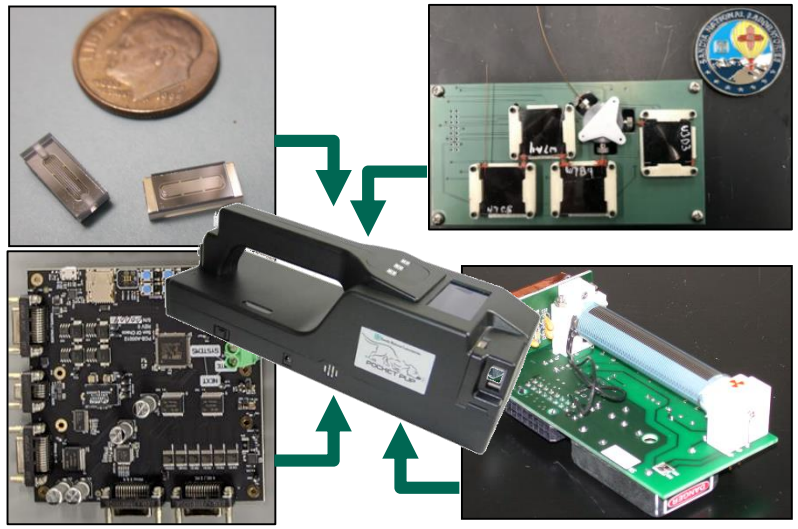
- Real-time multianalyte sensing
- Proteins, RNA, small molecule biomarkers in dermal interstitial fluid
- Early warning capabilities across populations for viral infections

POC(s): Ronen Polsky (rpolsky@sandia.gov), Phil Miller (prmille@sandia.gov)

VOC Biomarker Discovery

- Sampling methods for breath, skin volatiles, chyme
- State of the art laboratory equipment and expertise for biomarker discovery & ID
- Novel modality for rapid diagnostics

POC: Joshua Whiting (jjwhiti@sandia.gov)



Portable VOC Diagnostics technologies

- 2D Micro GC system
- Custom ion mobility spectrometer
- Custom low-SWaP electronics
- Chemical collector to improve selectivity

POC: Matthew Moorman (mmoorma@sandia.gov)

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Other Sandia Biosensing/Diagnostic technologies (Slide 2 of 2)

BaDx

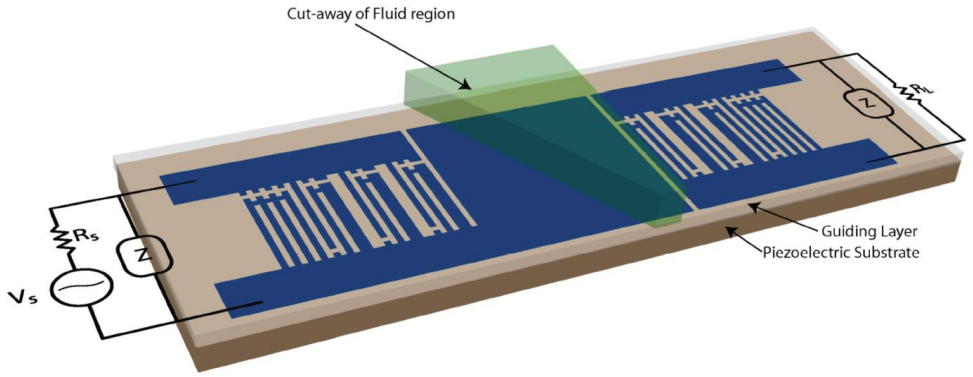
- No-power cartridge for culture-based anthrax detection & ID
- Self-sterilizing
- Adaptable to other pathogens

POC: Jason Harper (jcharpe@sandia.gov)

Surface Acoustic Wave Biosensors

- Sensitive, scalable, multi-mode detection (proteins, nucleic acids)
- Miniature low-power electronics
- Integrated sample prep

POC: Darren Branch (dwbranc@sandia.gov)



Collaborating with Sandia

Sandia *IS NOT* a funding agency!

- Sandia is a federally funded research and development center (FFRDC) owned by the US Department of Energy
- (Almost) all of Sandia's work is funded by federal government (DOE and non-DOE)
- Sandia works on full cost recovery

- Sandia has limited "internal funding" – Laboratory Directed Research & Development (LDRD)
  - LDRD is a congressionally-mandated program that allows National Labs to use a small percentage of funds from federal sponsors for internally directed R&D efforts
- Sandia dedicates some portion of these LDRD funds to seed collaborative projects with Academic Alliance partners

Academic Alliance LDRD funding is not an end in itself!

- The goal is to catalyze enduring, sustainable collaborations with external sponsorship, as well as talent development

Strategizing pathways to externally funded collaborations

University leads

- Work may be basic science, but relies on a unique Sandia capability
- Certain sponsors and mechanisms that Sandia can't be lead institution for (*e.g.* NSF, non-profits)

Sandia leads

- Basic or applied science
- Good fit to national lab mission and capabilities or existing program
- Sandia may rely on university partner for unique capabilities, *e.g.* BSL-3, hospital collaboration

Sponsor ecosystem - some are more suitable than others for national lab involvement

Rapid Point-of-Care Testing and Monitoring

https://diagnostics.roche.com/us/en/products/params/elecsys-anti-sars-cov-2.html

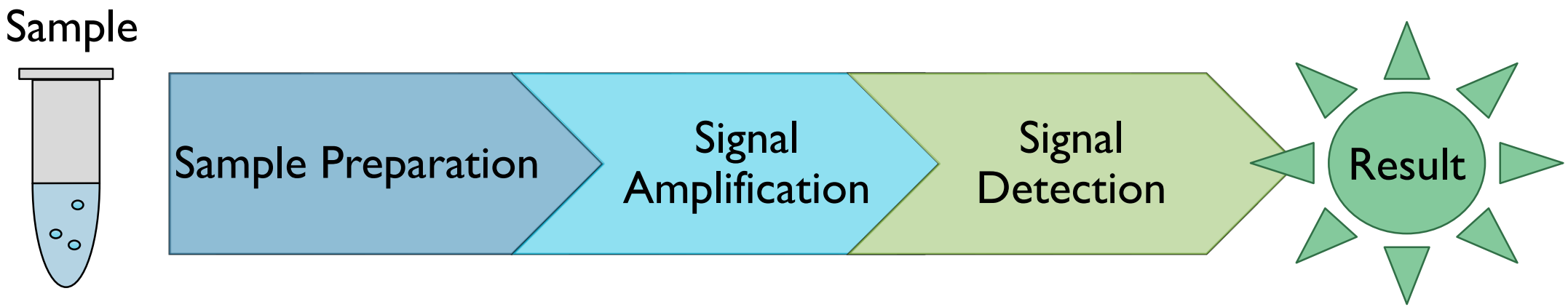
Jacqueline Linnes, PhD

Marta E. Gross Assistant Professor
Weldon School of Biomedical Engineering
Purdue University
jlinnes@purdue.edu

DIAGNOSTICS & SENSORS FOR FUTURE PANDEMIC RESPONSE

PURDUE UNIVERSITY

DIAGNOSTICS: SAMPLE-T0-RESULT DETECTION



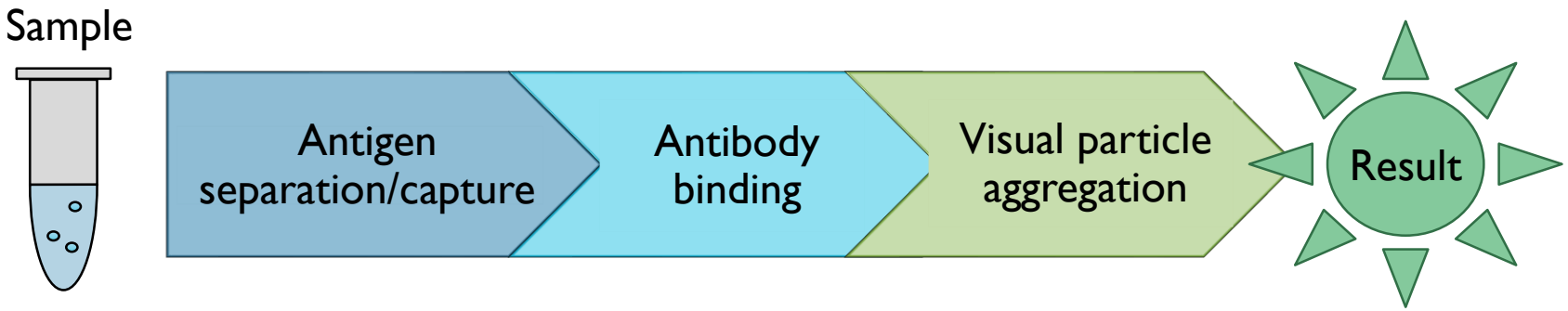
LABORATORY PCR-BASED TESTS

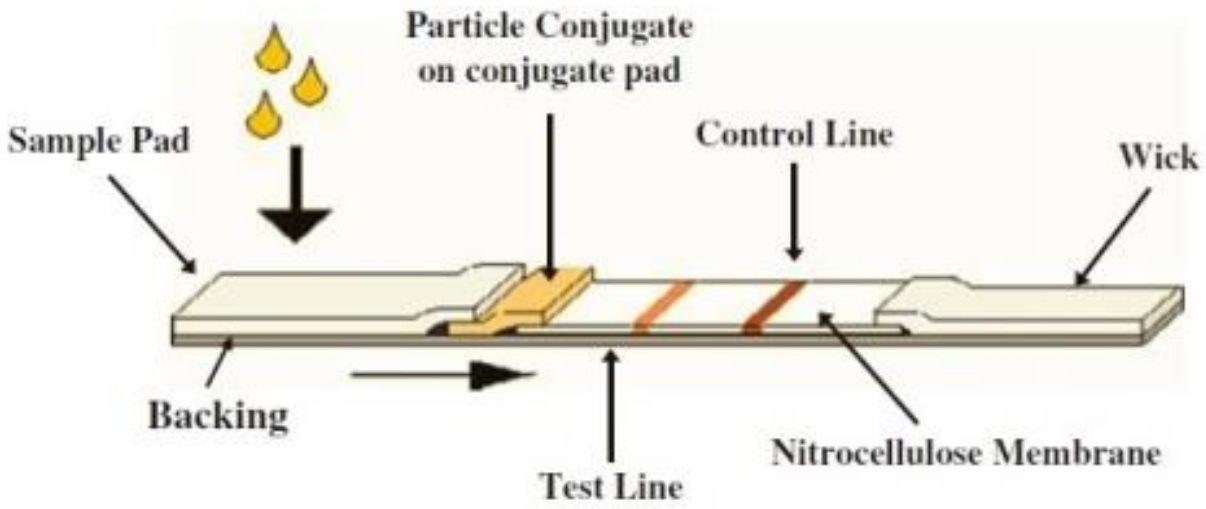
- Automated Detection
  - Multiplex samples and sample pooling
  - High throughput

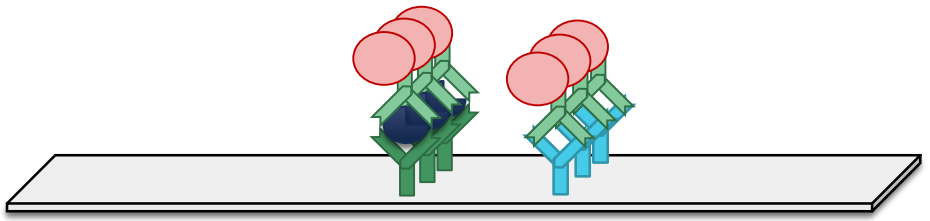
- Cons:
  - Samples must be shipped to lab
  - Results not immediate

https://helpstpauls.com/2020/03/24/covid-19/virology-laboratory-at-st-pauls-first-in-canada-to-have-new-high-throughput-covid-19-testing-capability

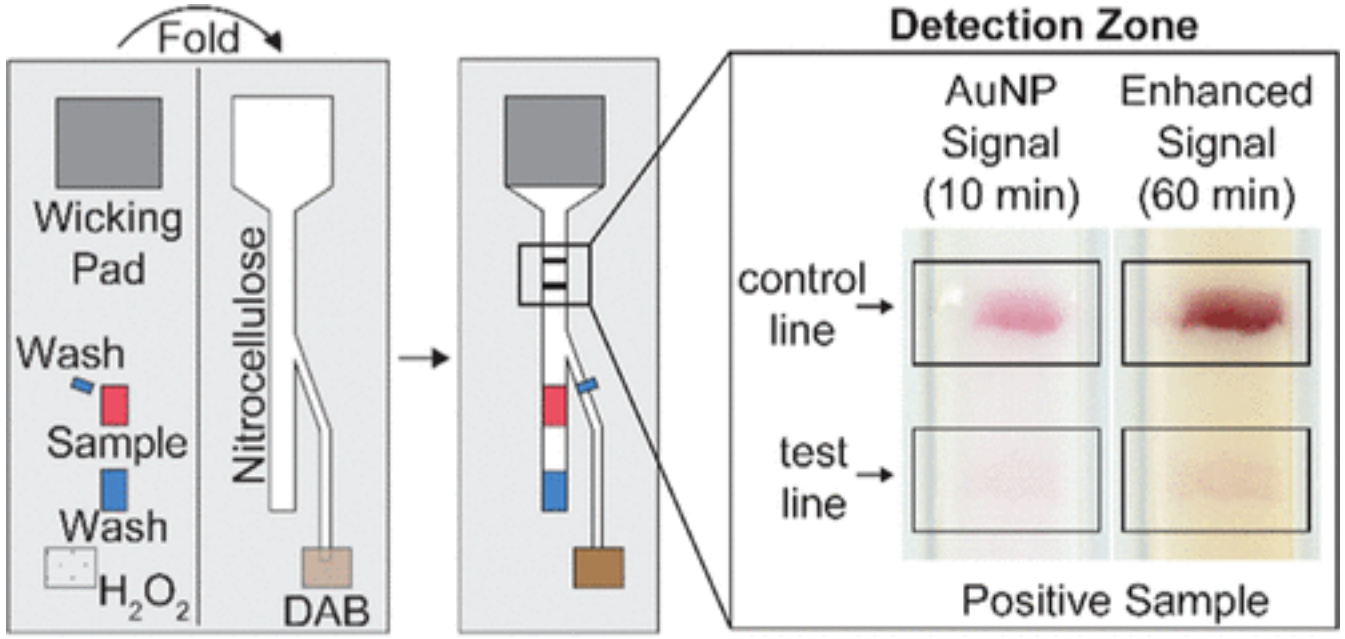
RAPID DIAGNOSTIC TESTS (ANTIGEN/ANTIBODY TESTS)

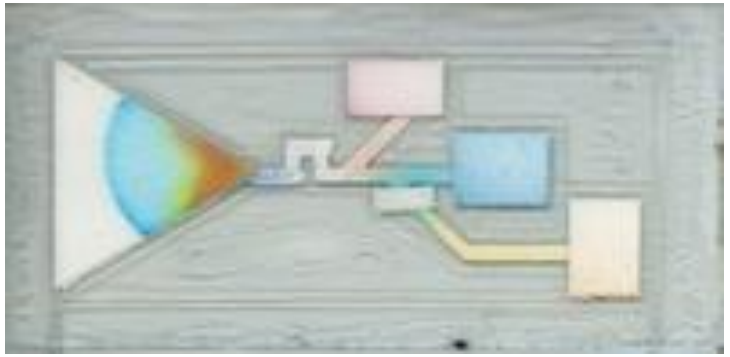




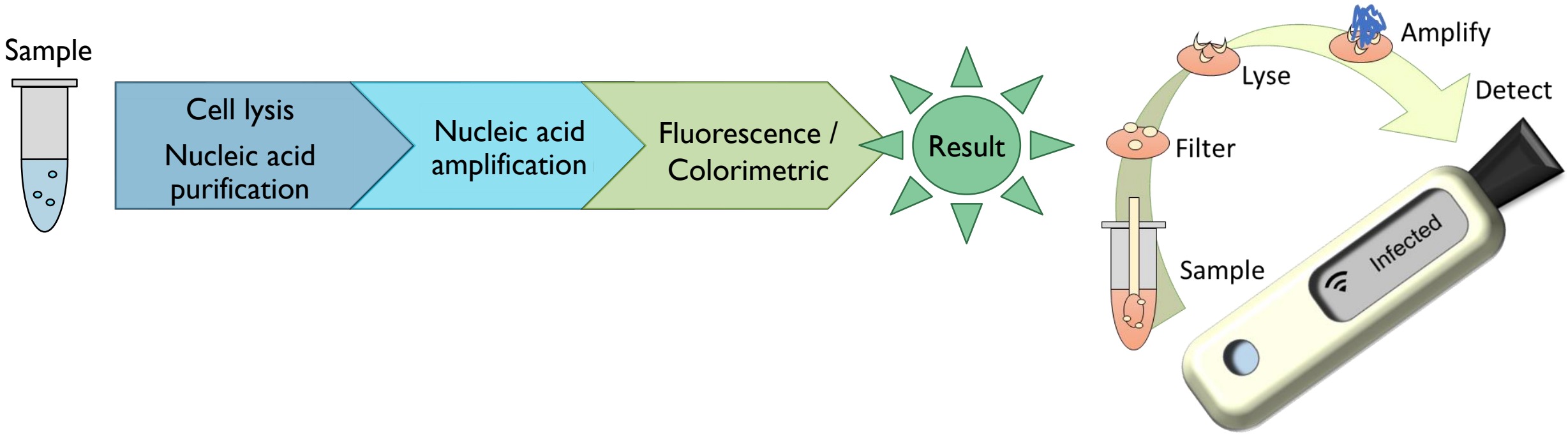


SIGNAL ENHANCED ANTIGEN/ANTIBODY TESTS



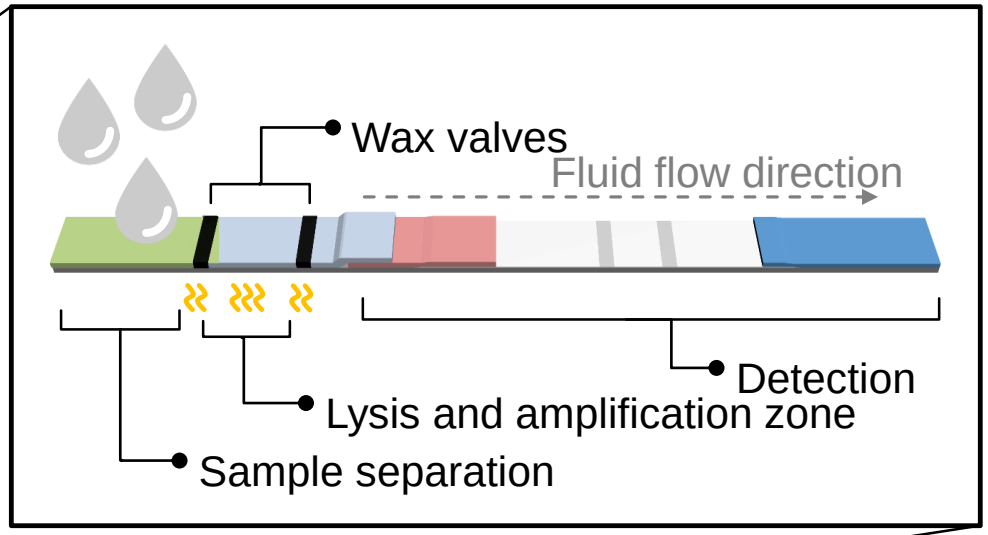


MOLECULAR DIAGNOSTICS



PAPER-BASED MOLECULAR DIAGNOSTICS

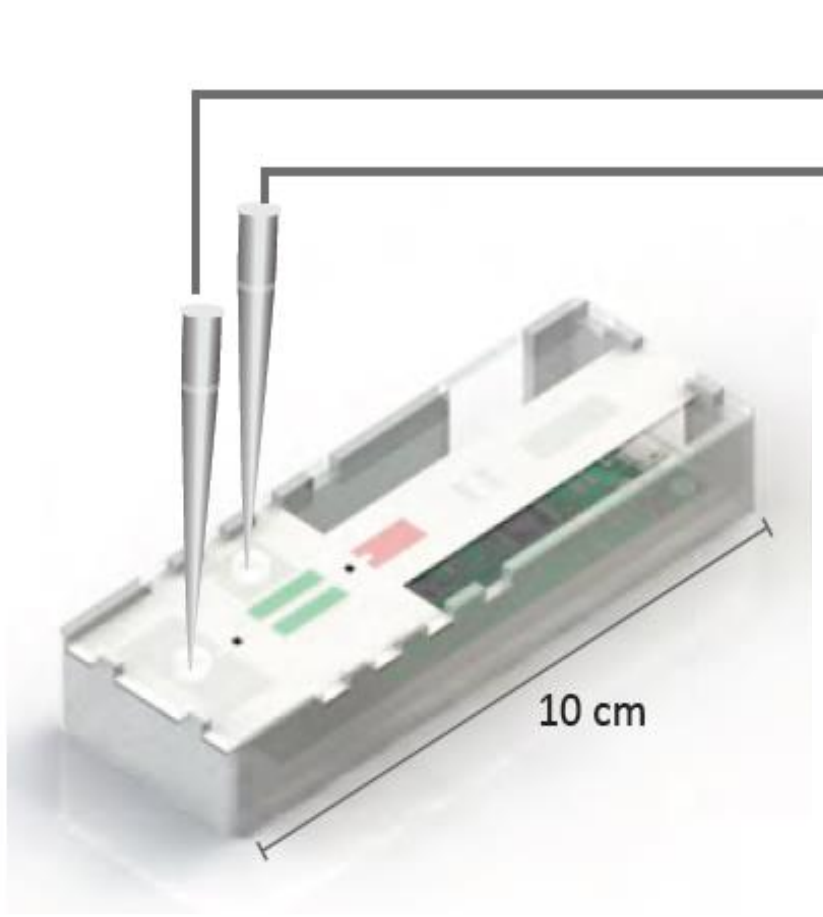
Combine simplicity and portability of handheld rapid tests with sensitivity and specificity of lab-based nucleic acid diagnostics

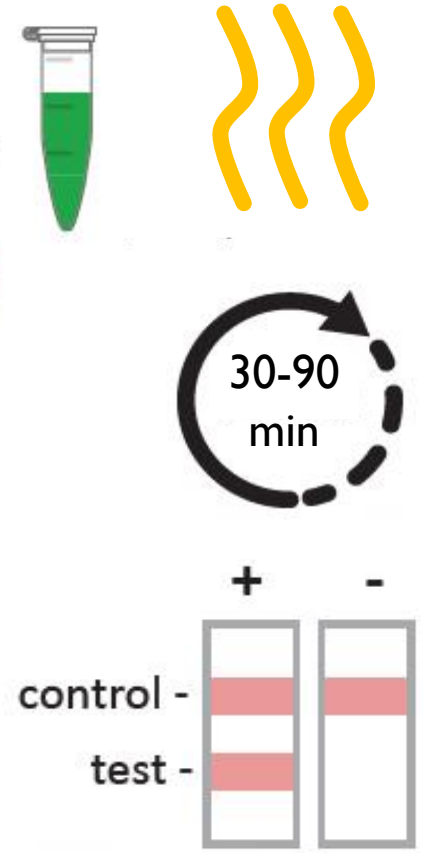


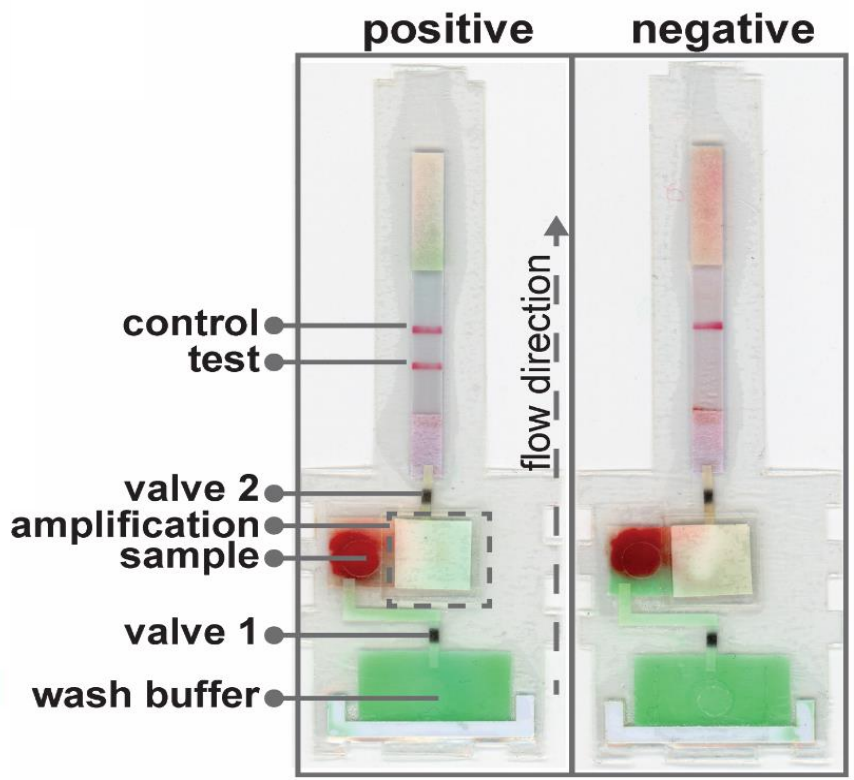
US20190126270A WO2017184665A1

Phillips *et al.*, *Lab on a Chip* 2016 Byers *et al.*, *Analyst* 2020 Phillips and Moehling *et al.*, *Lab on a Chip* 2019

USER STEPS

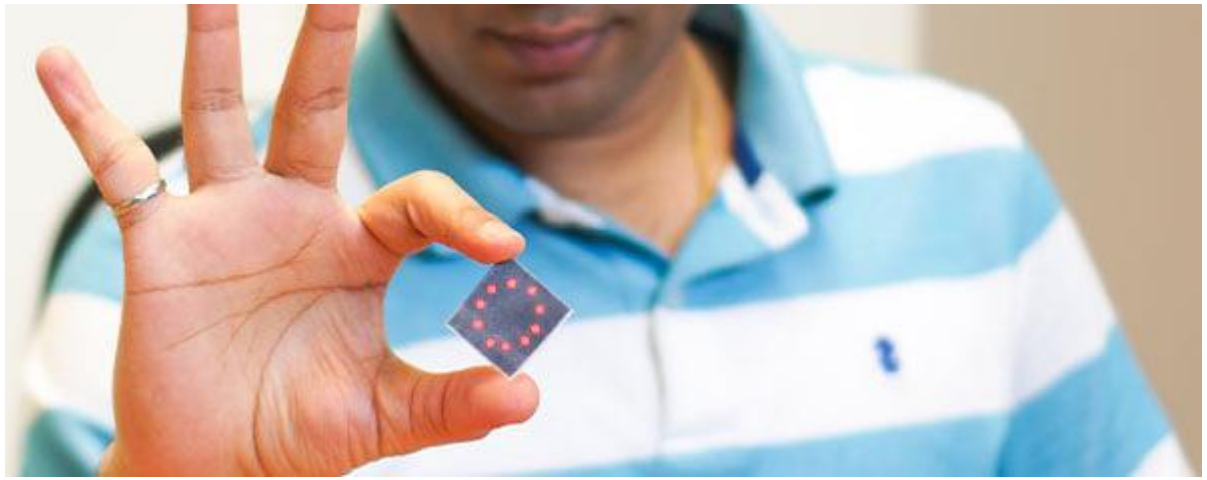






Phillips and Moehling et al. *Lab on a Chip* 2019

PAPER-BASED DIAGNOSTICS FOR RESPIRATORY INFECTIONS



Mohit Verma

COVID-19 Test: Purdue University, Raytheon BBN Technologies, a Raytheon Technologies company, PortaScience Inc., Cortex Design Inc., and LaDuca LLC

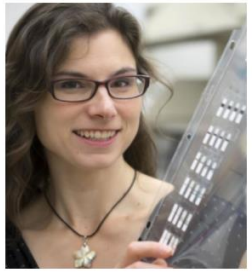
https://www.purdue.edu/newsroom/releases/2020/Q2/purdue-university,-raytheon-bbn-technologies,-portascience,-cortex-design,-and-laduca-partner-on-covid-19-point-of-care-test-kit.html

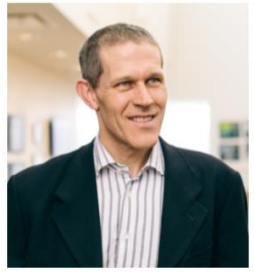
PORTABLE SMARTPHONE PLATFORM

OmniVis

Katherine Clayton

Tamara Kinzer-Ursem

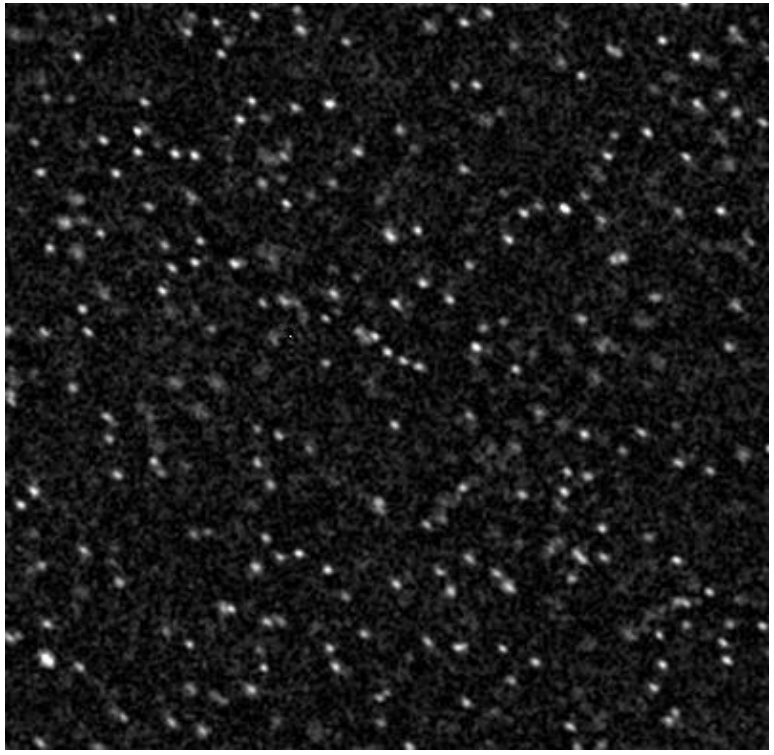




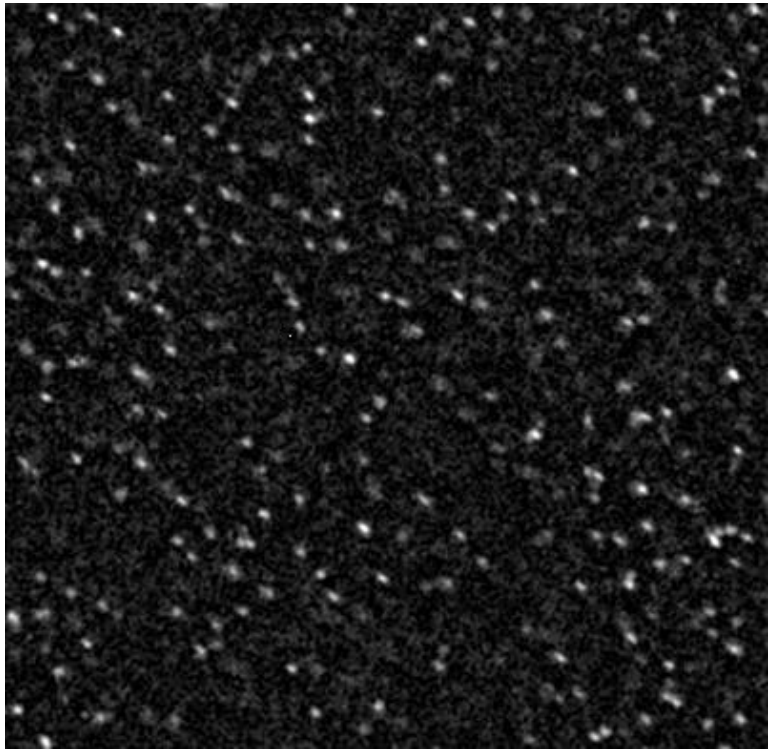
Steven Wereley

Conflict of Interest Warning: I am a Co-Founder of OmniVis, Inc.

VISCOSITY CHANGES WITH AMPLIFIED NUCLEIC ACID

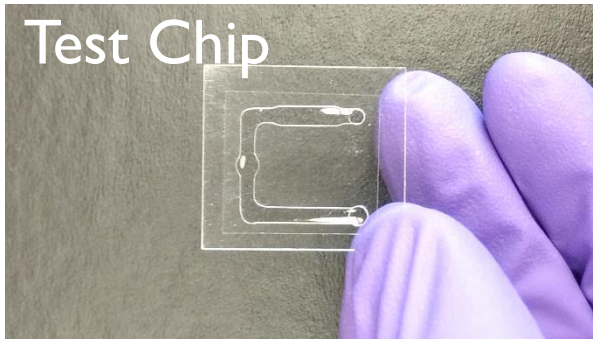


Low Diffusivity:
Pathogen Detected



High Diffusivity:
No Pathogen

SMARTPHONE DIFFUSOMETER



Camera + Computer

I: Add sample

II: Heat for 30 min

III: Add nanoparticles

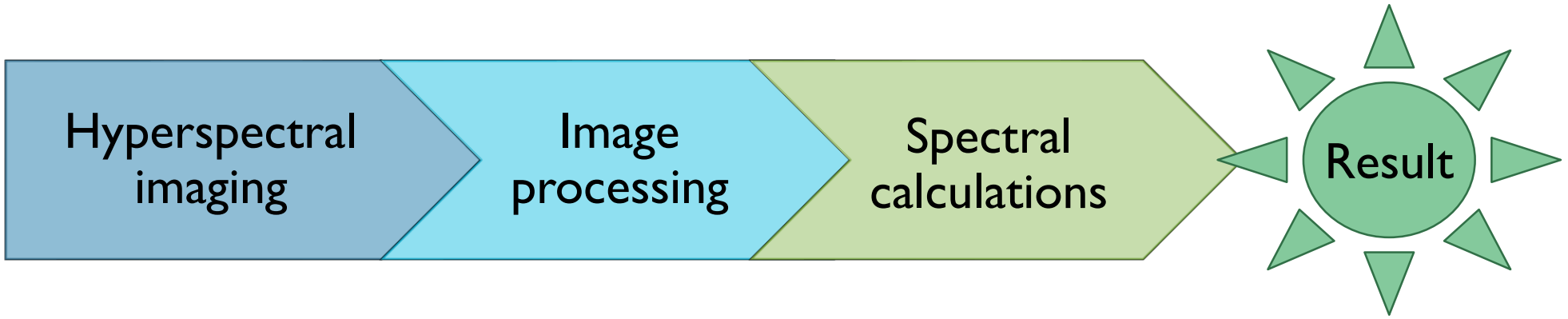
IV: Deposit into chip

V: Image & analyze

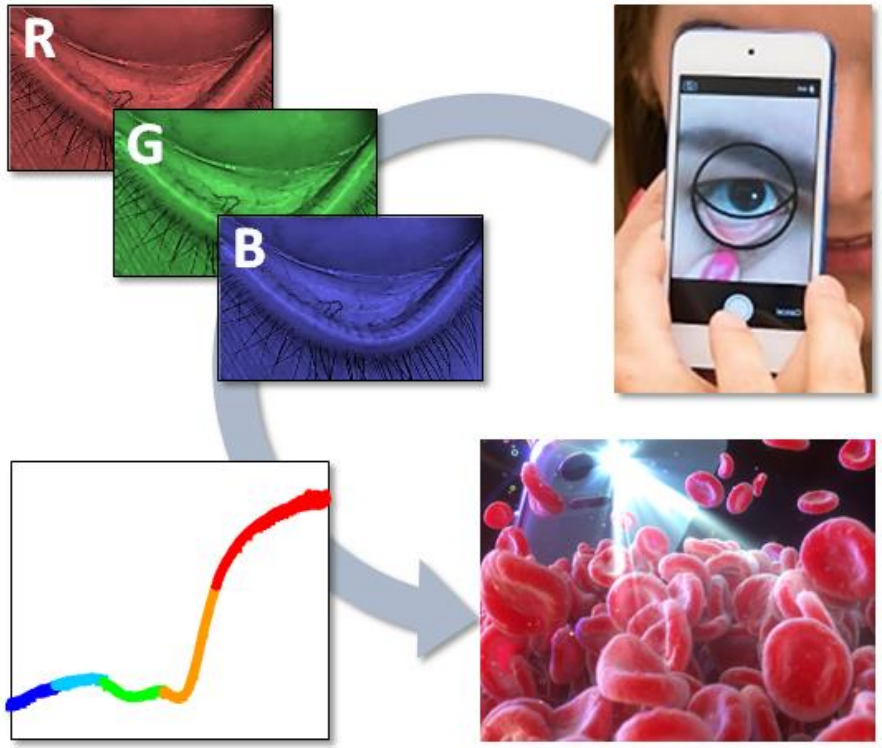
Taylor Moehling and DongHoon Lee et al. *Biosensors and Bioelectronics* 2020

WEARABLE ELECTROPHYSIOLOGY DEVICES

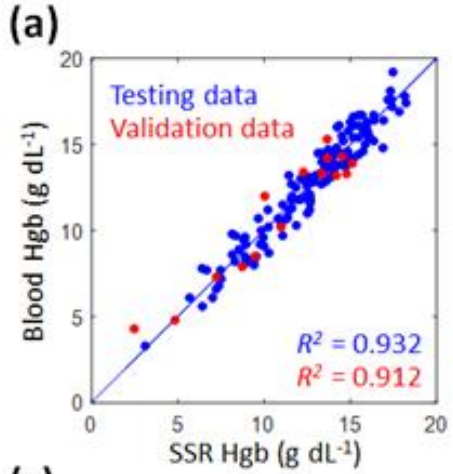
Sample

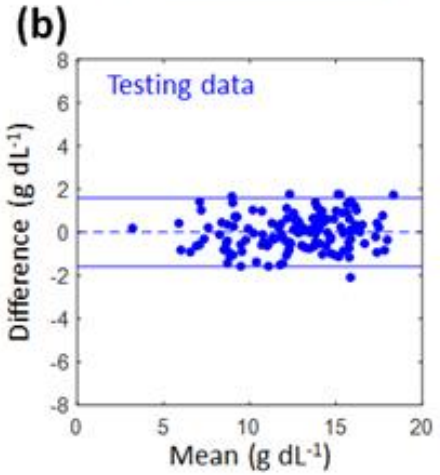


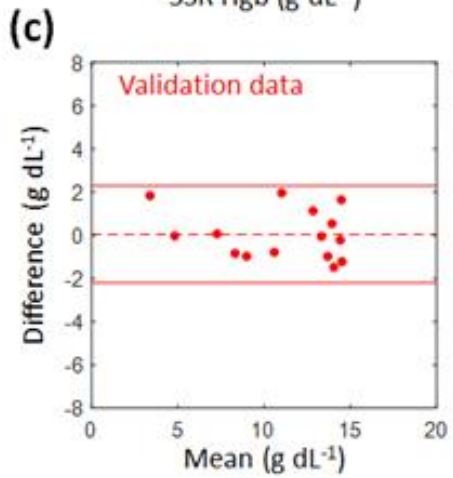
M-HEALTH SPECTROSCOPY – NONINVASIVE BLOOD ANALYSIS



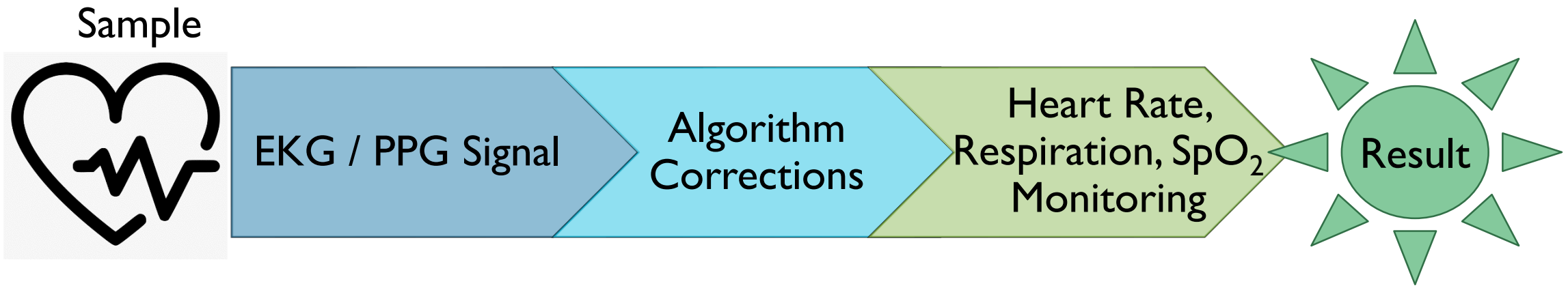
Young Kim
Natural and Biological Photonics Laboratory







WEARABLE ELECTROPHYSIOLOGY DEVICES



WEARABLES FOR INFECTION MONITORING

(Purdue University photo/John Underwood)

Craig Goergen

CardioVascular Imaging Research Laboratory

Accurate, Accessible Testing for the Whole Community

Jacqueline Linnes, PhD
jlinnes@purdue.edu

https://www.elmhurst.edu/blog/what-is-community-health/

Thoughts on Pathogen Detection for Emerging Threats

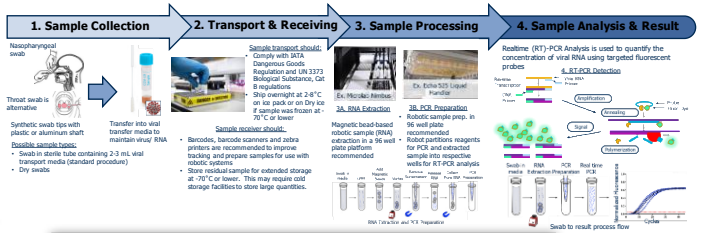
Michael Shannon, Ph.D.
Mike Farrell, Ph.D.
True Merrill, Ph.D.
Nick Speller, Ph.D.
Miles Paca

05NOV2020

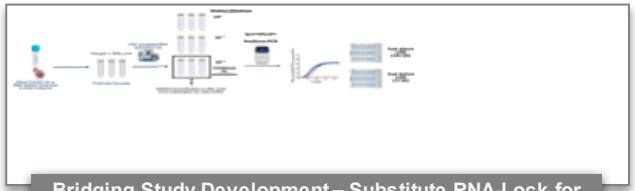
GTRI developed a saliva-based COVID-19 test

GOAL: develop a *Georgia*-based, supply chain-stable, affordable, molecular real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in saliva specimens

Technical Pedigree



Kit Production Capability Development



Bridging Study Development – Substitute RNA-Lock for Viral Transport Medium (VTM)

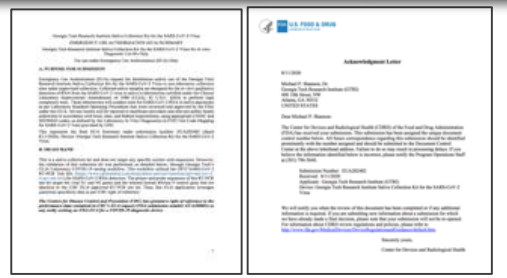
Georgia solutions to Georgia problems

Omega Bio-tek
Norcross, GA

Omni International
Kennesaw, GA

FDA Emergency Use Authorization (pending final approval):

Georgia Tech Research Institute Saliva Collection Kit for the SARS-CoV-2 Virus
Georgia Tech SARS-CoV-2 Virus Molecular Diagnostic Test (GT COVID-19 Saliva Test)



Clinical Validation with Georgia DPH

Validation Plan

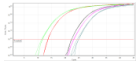
Nasopharyngeal Swab Kit

Saliva Collection Kit

Collect both sample types from same patient

Extraction

Reverse Transcription Real-Time PCR



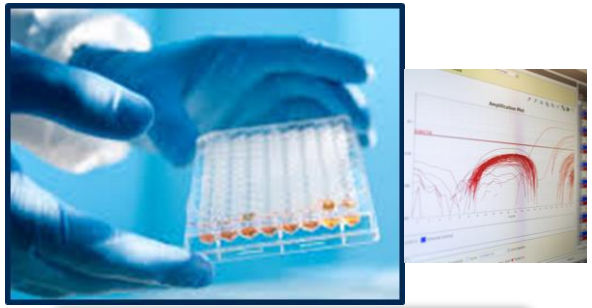
DPH IRB Approval

Seeking 30 negative samples and 30 positive samples for comparison

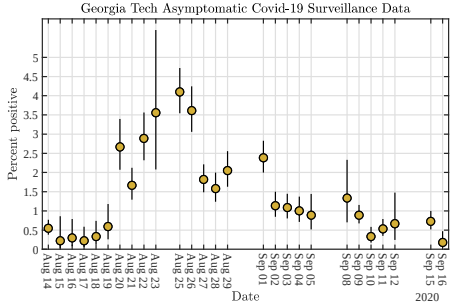
Our Interests – Coupling Speed with Gold Standard Assays for Pathogen Detection

Focus across pathogen families

Novel Specimen Collection



Extractionless PCR



Asymptomatic Testing at Scale

Innovation in Point of Care

Attacking current gaps by creating capabilities to improve testing speed, scale and fidelity

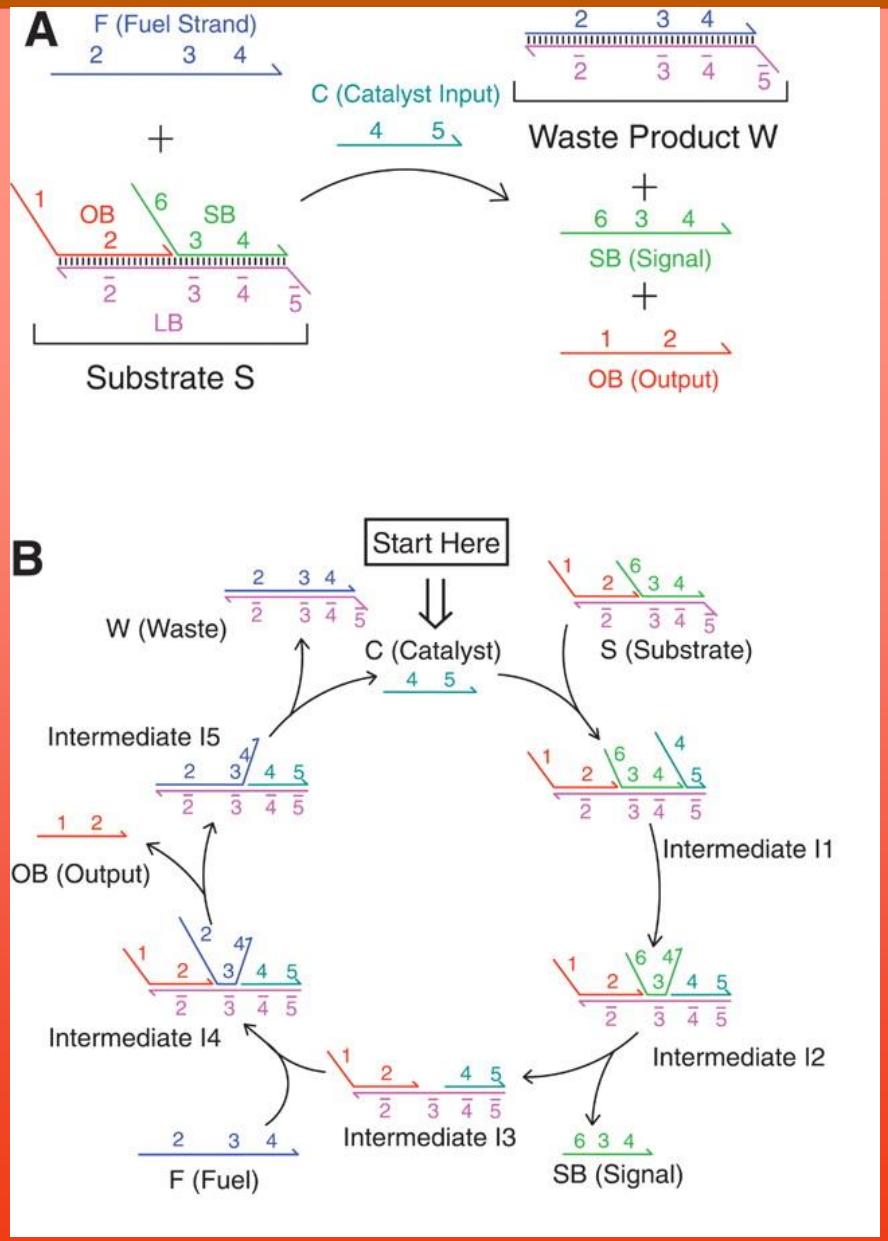
Georgia Tech Research Institute

Isothermal Amplification for the Detection of SARS-CoV-2

Andrew D. Ellington
Center for Systems and Synthetic Biology
University of Texas at Austin

Sandia, November 5, 2020

How to make computers with carbon, rather than silicon



Zhang et al. (2007) Science 318:1121

Erik Winfree

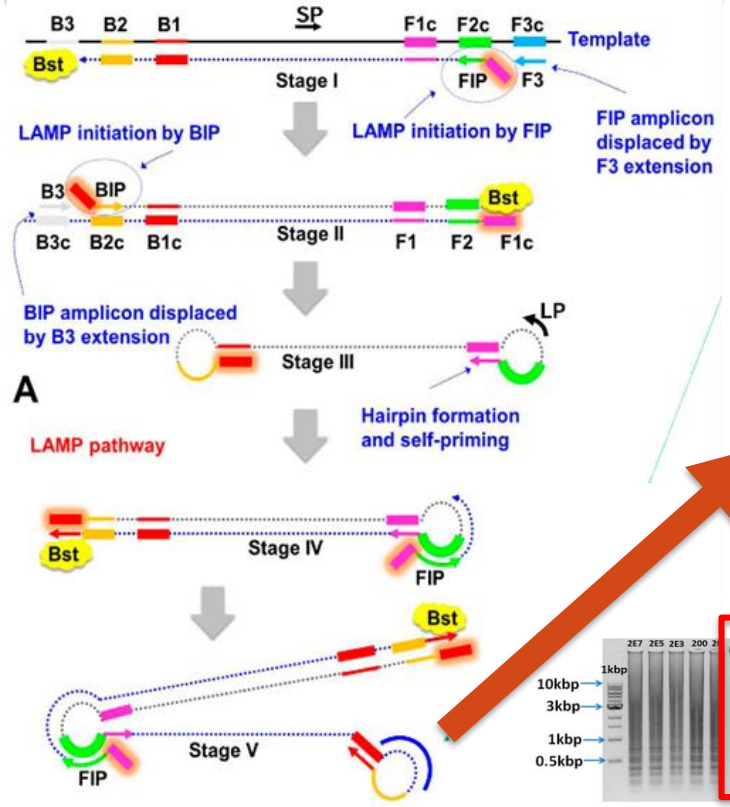
Niles Pierce

Peng Yin

LAMP-OSD

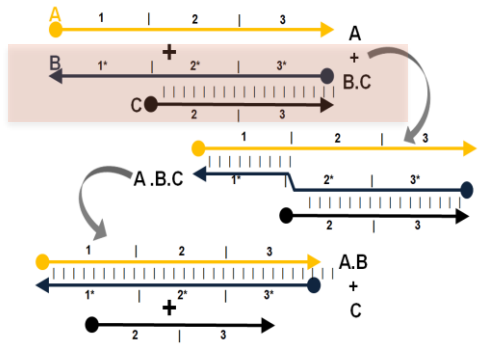
DNA/RNA amplification

LAMP



Signal processing

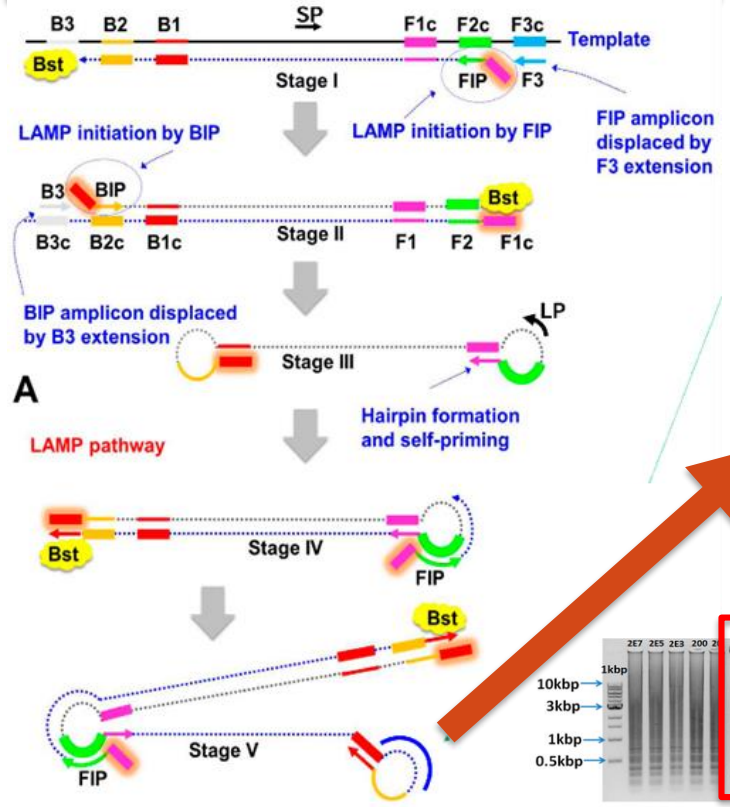
Toehold-mediated strand exchange computation probes



LAMP-OSD

DNA/RNA amplification

LAMP



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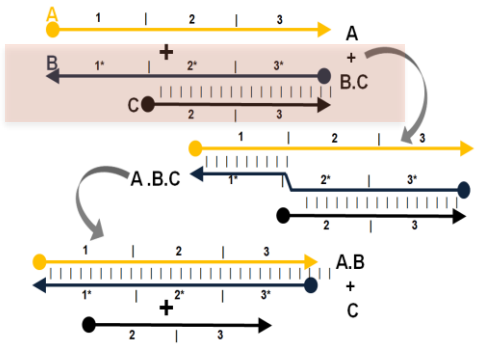
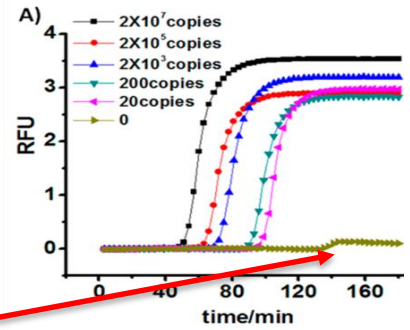
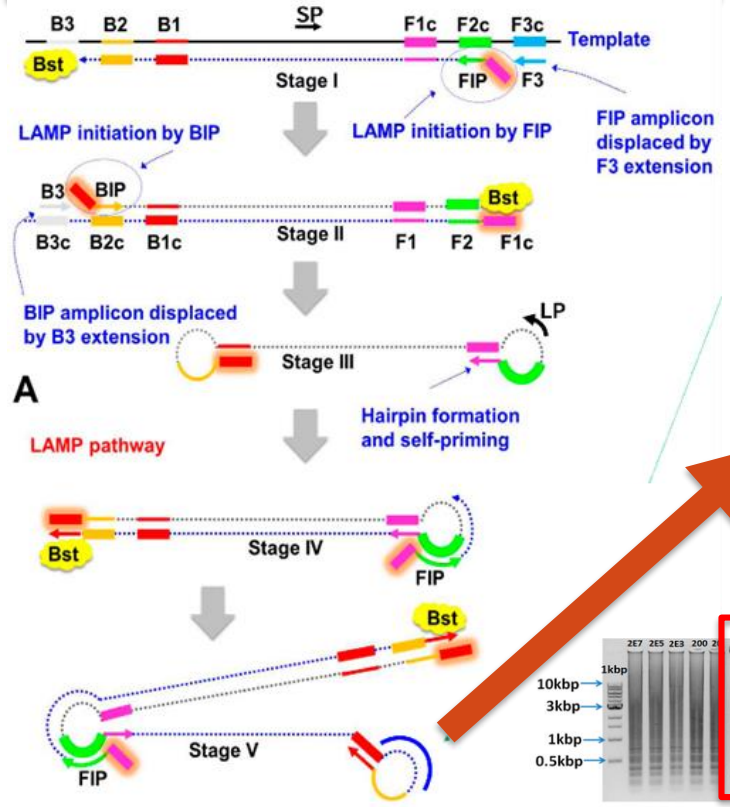




LAMP-OSD

DNA/RNA amplification

LAMP



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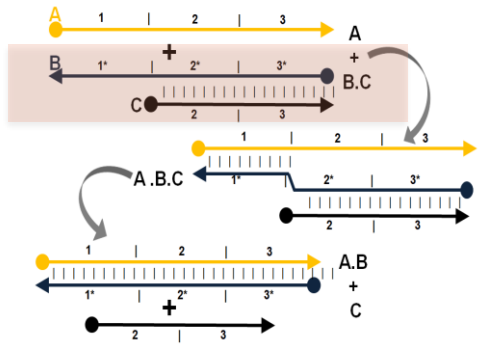
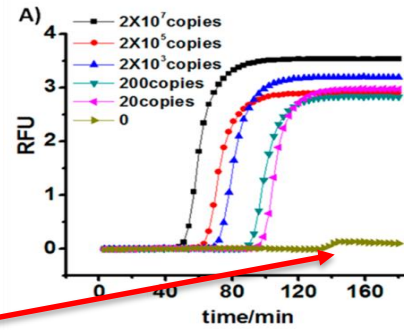
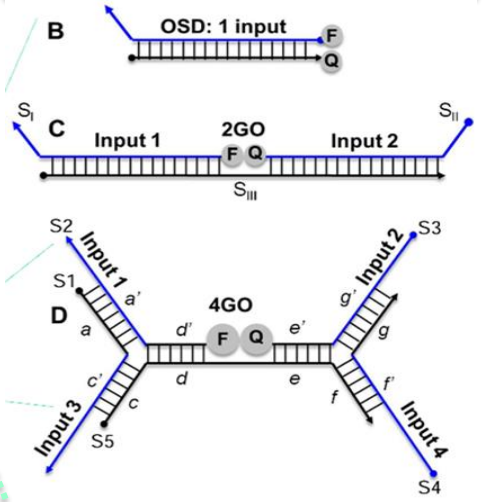




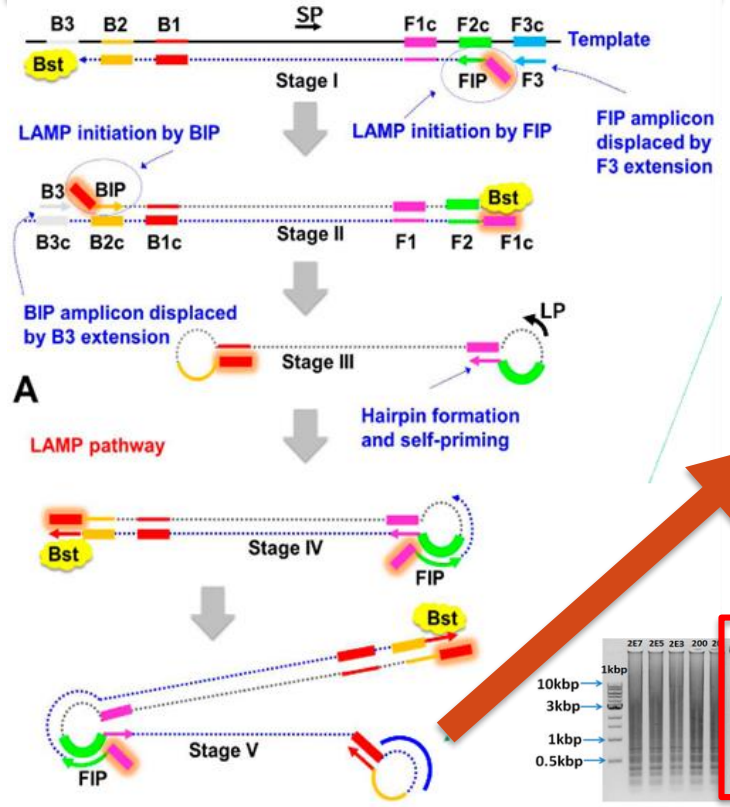
One or multiple amplicon processing



LAMP-OSD

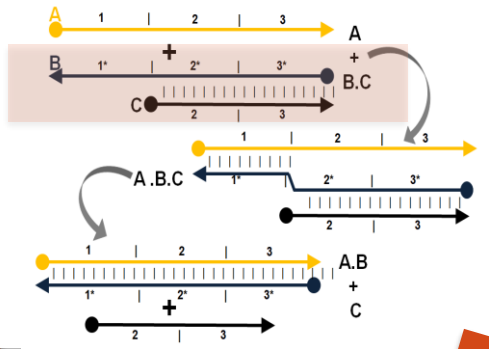
DNA/RNA amplification

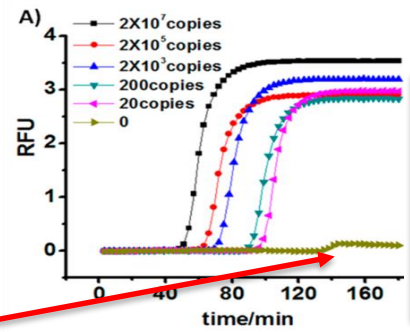
LAMP



Signal processing

Toehold-mediated strand exchange computation probes





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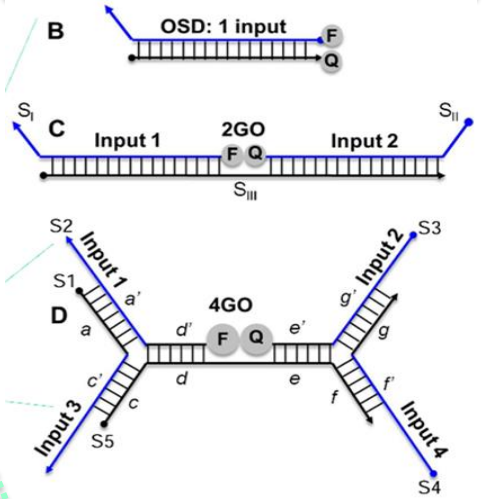


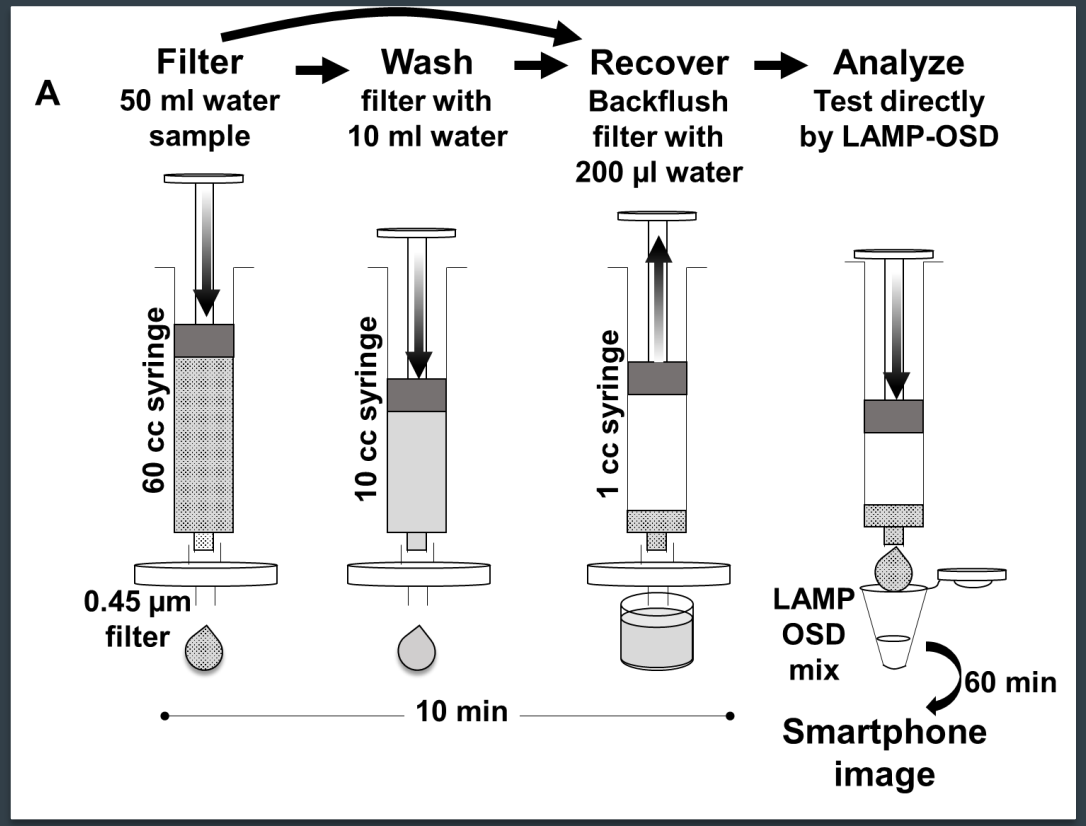
Visual readout

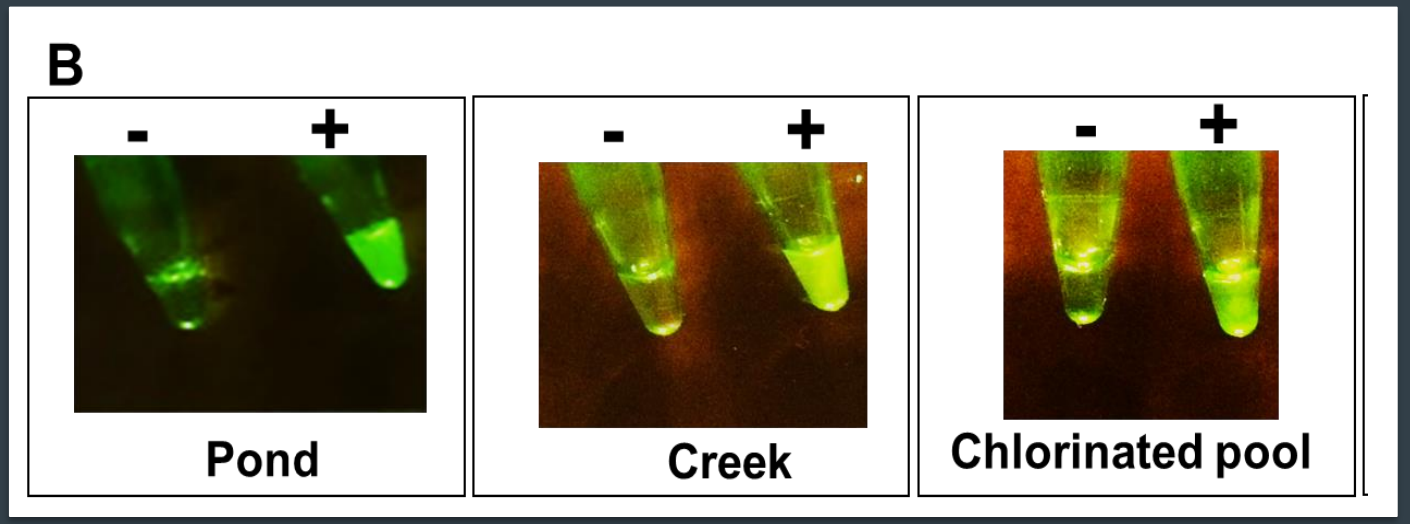
Cellphone

Dipstick

We nonetheless had some confidence we could make a pretty good mousetrap

Detection of human fecal contamination in recreational waters



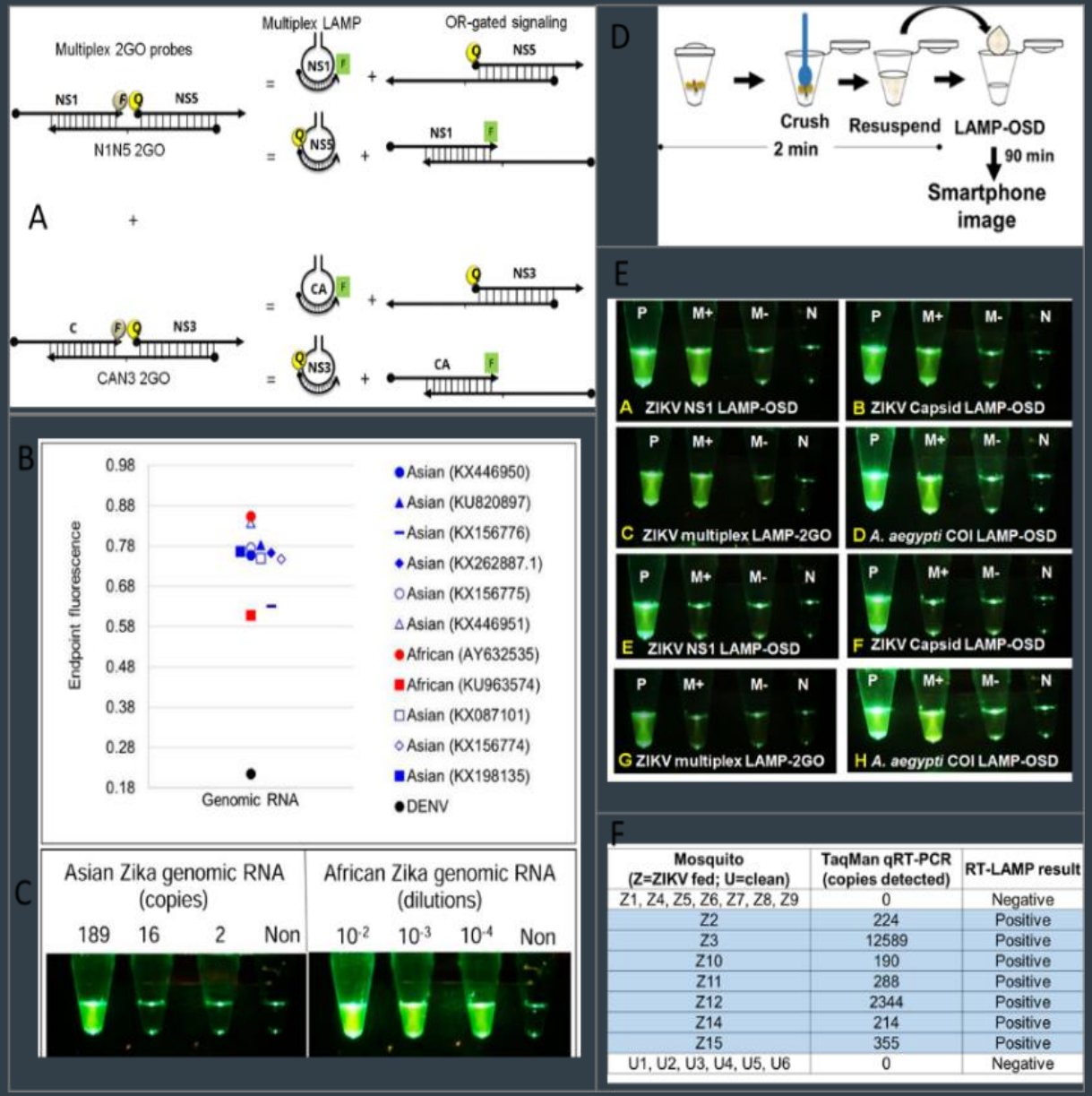


Bacteroides HF183 LAMP-OSD

Especially a good mousetrap for viruses!

Or-gated probe captures multiple amplicons into one signal

Use of degenerate primers captures phylogenetically wide range of Zika viruses



F

Mosquito (Z=ZIKV fed; U=clean)	TaqMan qRT-PCR (copies detected)	RT-LAMP result
Z1, Z4, Z5, Z6, Z7, Z8, Z9	0	Negative
Z2	224	Positive
Z3	12589	Positive
Z10	190	Positive
Z11	288	Positive
Z12	2344	Positive
Z14	214	Positive
Z15	355	Positive
U1, U2, U3, U4, U5, U6	0	Negative

Direct detection from mosquito guts

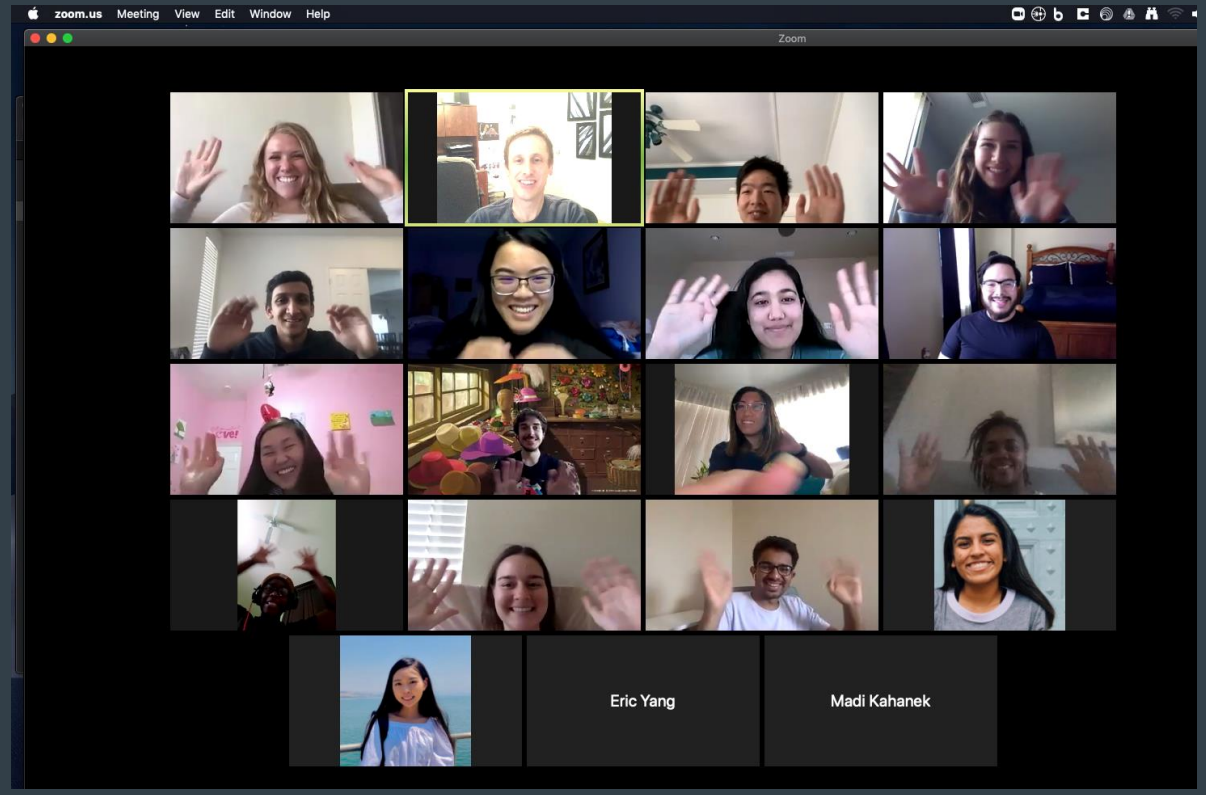
Cell phone image detection of individual infected mosquitoes

Results align with qPCR

Bhadra et al. (2018), *Viruses* 10:714;
Bhadra et al. (2018), *PLoS Negl Trop Dis* 12:e0006671

Involving undergraduates in the crisis that besets us all

- Feb 24, DIY Diagnostics Mentors and Students vote to change spring curriculum
  - New spring students implement COVID-19 CDC qPCR Dx
  - Experienced students form development team for COVID-19 LAMP-OSD Dx
- Feb 29, Existing COVID LAMP assays pulled from literature and ordered
- March 2, First successful demonstration of CDC qPCR
- March 4, screening of known primer sets begin
- March 11, OSDs designed and ordered
- March 13, campus closed, project transferred to Ellington Lab



Author	Preprint Date	Gene Target	Temp	Validation
El-Tholoth et al.	2020.02.16	ORF1ab	63	Synthesized DNA
Lamb et al. (Beaumont)	2020.02.19	ORF1a (3043-3301)	65	Synthetic Patient Samples
Yu et al.	2020.02.20	ORF1ab	63	Patient Samples
Zhang et al.(NEB)	2020.02.26	ORF1a-A	65	Patient samples
Zhang et al. (NEB)	2020.02.26	ORF1a-B	65	Patient samples
Zhang et al. (NEB)	2020.02.26	ORF1a-C	65	Patient samples
Zhang et al. (NEB)	2020.02.26	Gene N-A	65	Patient samples
Zhang et al. (NEB)	2020.02.26	Gene N-B	65	Patient samples
Yang et al.	2020.03.02	ORF1ab	63	Patient samples
Yang et al.	2020.03.02	N	63	Patient samples
Yang et al.	2020.03.02	E	63	Patient samples

As usual, primer choice in LAMP is key

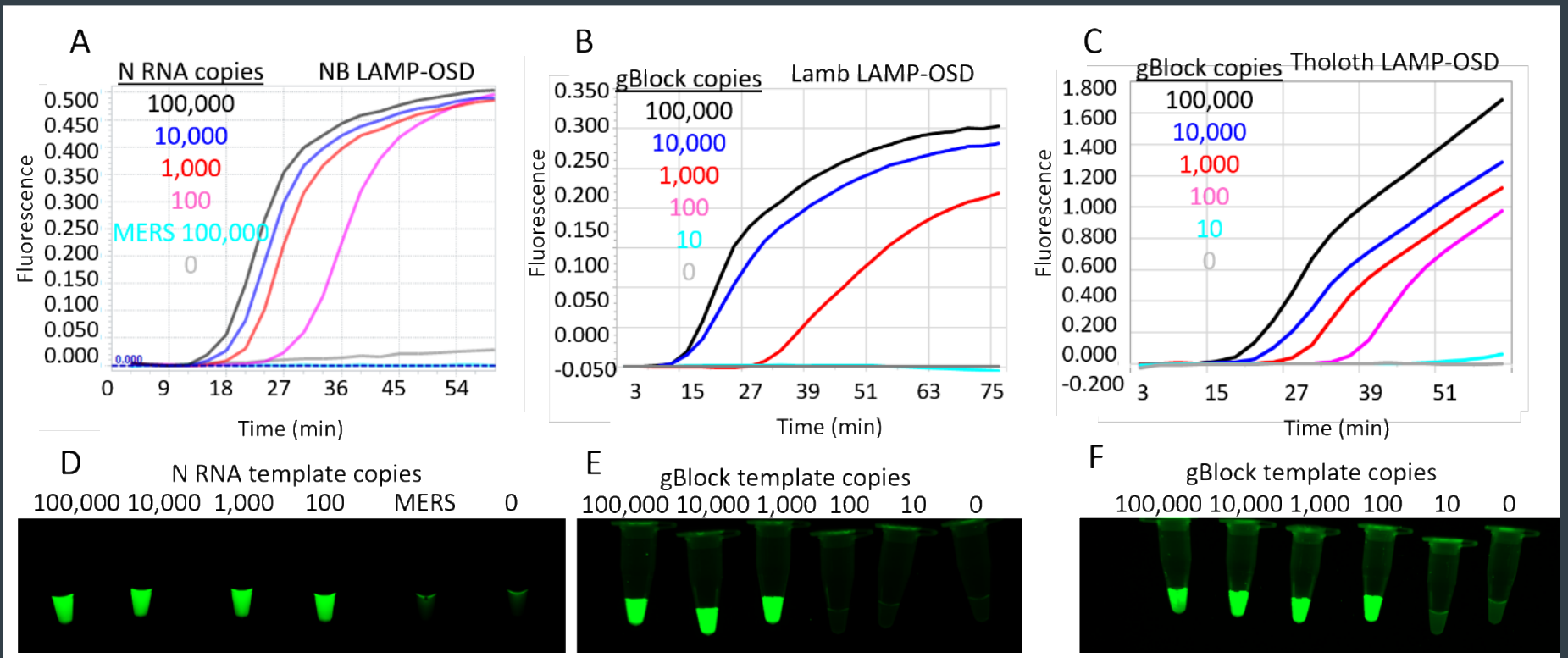


Figure 2. SARS-CoV-2 LAMP-OSD assays. OSD fluorescence measured in real-time during LAMP amplification for NB (A), 5 primer Lamb (B), and Tholoth (C) LAMP-OSD assays are depicted as amplification curves. Presence or absence of OSD fluorescence visually observed at assay endpoint for NB (D), Lamb (E), and Tholoth (F) LAMP-OSD assays are depicted as images of reaction tubes. NB LAMP-OSD assays were seeded with indicated copies of SARS-CoV-2 N RNA or MERS-CoV N RNA or no templates. Lamb and Tholoth LAMP-OSD assays were seeded with indicated copies of gBlock DNA templates.

We also have newer primer sets that reduce detection time to 20’ - 30’

From: https://www.biorxiv.org/content/10.1101/2020.04.13.039941v3.full.pdf

Working towards a true POC assay

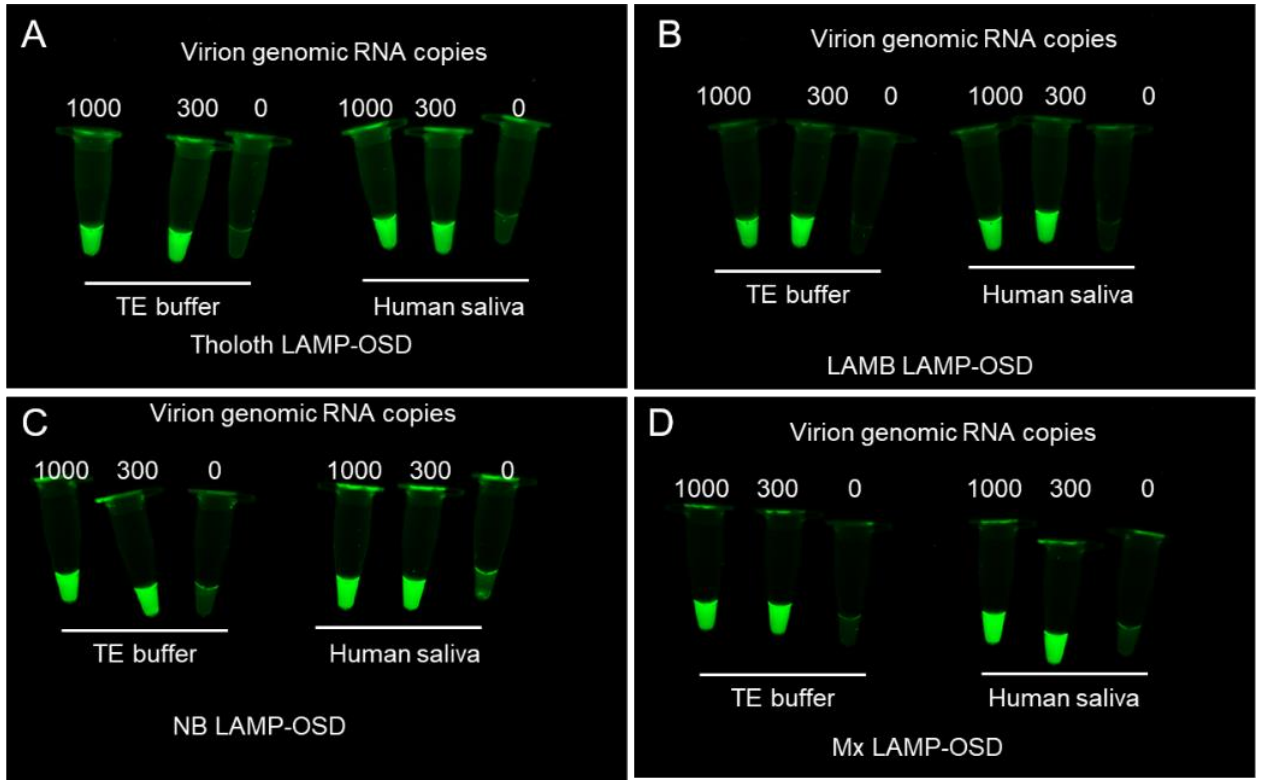


Figure 6. LAMP-OSD analysis of human saliva containing SARS-CoV-2 virions. Indicated virion amounts were spiked in TE buffer or human saliva and added to individual or multiplex (Mx) LAMP-OSD assays. Endpoint images of OSD fluorescence are depicted for Tholoth (A), 6 primer LAMB (B), and NB (C) individual LAMP-OSD assays and Tholoth+NB Mx LAMP-OSD assays (D).

- Spike inactivated virions into saliva
- Add to LAMP-OSD assay
- That's it. Really
- Sensitivity could be better … but keep this Figure in mind going forward

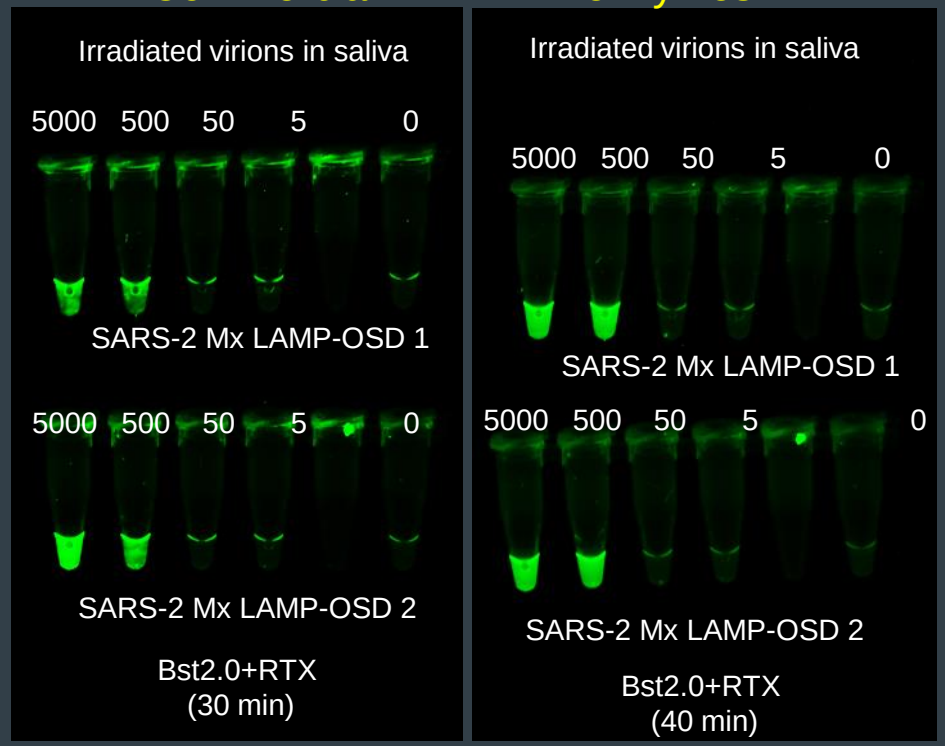
https://www.biorxiv.org/content/10.1101/2020.04.13.039941v3

Direct saliva analysis within 30 to 40 min

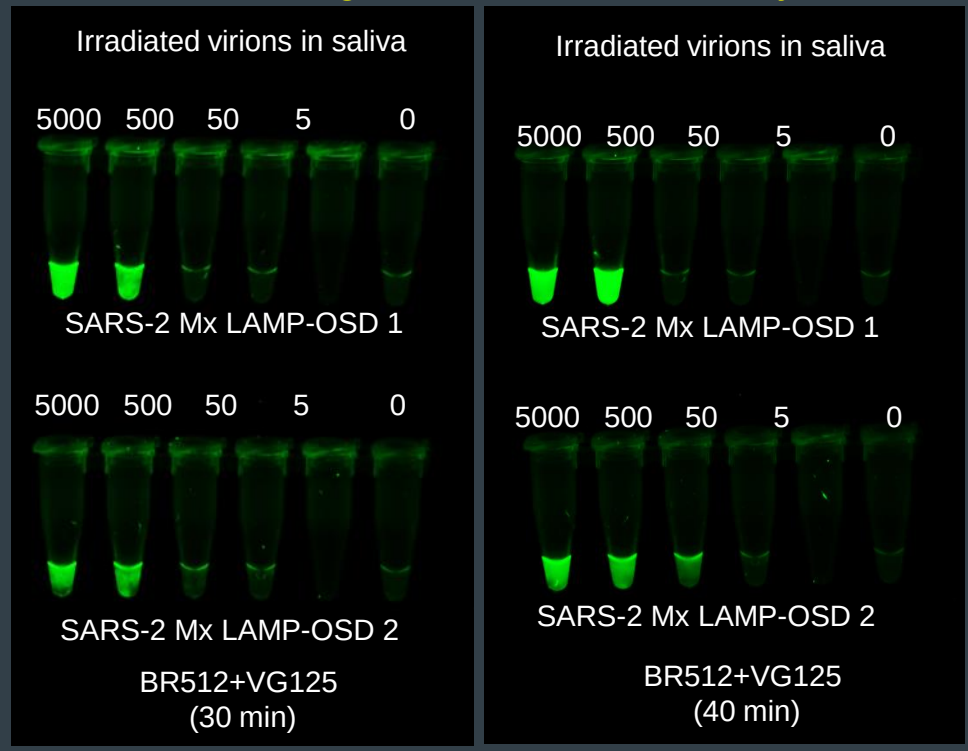
1. 3 µL of irradiated SARS- CoV-2 virions and saliva directly added to a total of 25 µL multiplex LAMP-OSD reactions
2. Reactions incubated at 65 °C for 30 to 40 min
3. OSD fluorescence imaged at endpoint

Mx LAMP-OSD 1 = one tube NB and Lamb6 multiplex assay
Mx LAMP-OSD 2 = one tube NB and Tholoth multiplex assay

Commercial RT-LAMP enzymes



In-house engineered RT-LAMP enzymes



The addition of in-house engineered enzymes improves performance

	SARS-CoV-2 DETECTR	SARS-CoV-2 SHERLOCK	CDC SARS-CoV2 qRT-PCR	RT-LAMP-OSD
Target	N gene & E gene (N gene gRNA compatible with CDC N2 amplicon, E gene compatible with WHO protocol)	S gene & Orf1ab gene	N-gene (3 amplicons)	Multiplex: ORF1ab, N gene
Sample control	RNase P	None	RNase P	Gapd
Limit of Detection	10-50 copies/µl input	10-100 copies/µl input	1-3.16 copies/µL input	2-20 copies/µL input (total 5 µL)
Assay reaction time	~40 min	~60 min	~120 minutes	60-90 min
Assay components	RT-LAMP (62°C, 30 min) Cas12 (37°C, 10 min) Lateral flow (RT, 2 min)	RT-RPA (42°C, 25 min) IVT + Cas13 (37°C, 30 min) Lateral flow (RT, 2 min)	UDG digestion (25°C, 2 min), reverse transcription (50°C, 15 min), denature (95°C, 2 min) amplification, (95°C, 3 sec; 55°C 30 sec; 45 cycles)	One pot RT-LAMP-OSD using either commercial enzymes Bst2.0 + RTx or using open source enzymes Bst-LF + FeRT
Heavy instrumentation required	No	No	Yes	No

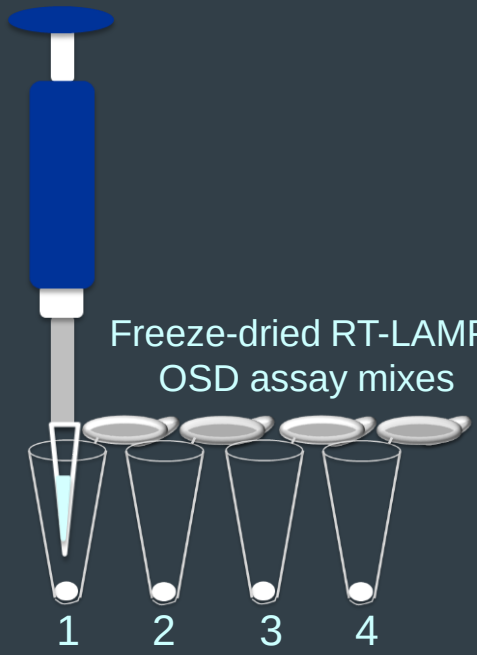
Detectr, Sherlock, and qRT-PCR metrics adapted from https://mammoth.bio/wp-content/uploads/2020/04/200423-A-protocol-for-rapid-detection-of-SARS-CoV-2-using-CRISPR-diagnostics_3.pdf

Cost estimate for SARS-CoV-2 test

Rough estimates based on market cost of small batch reagents

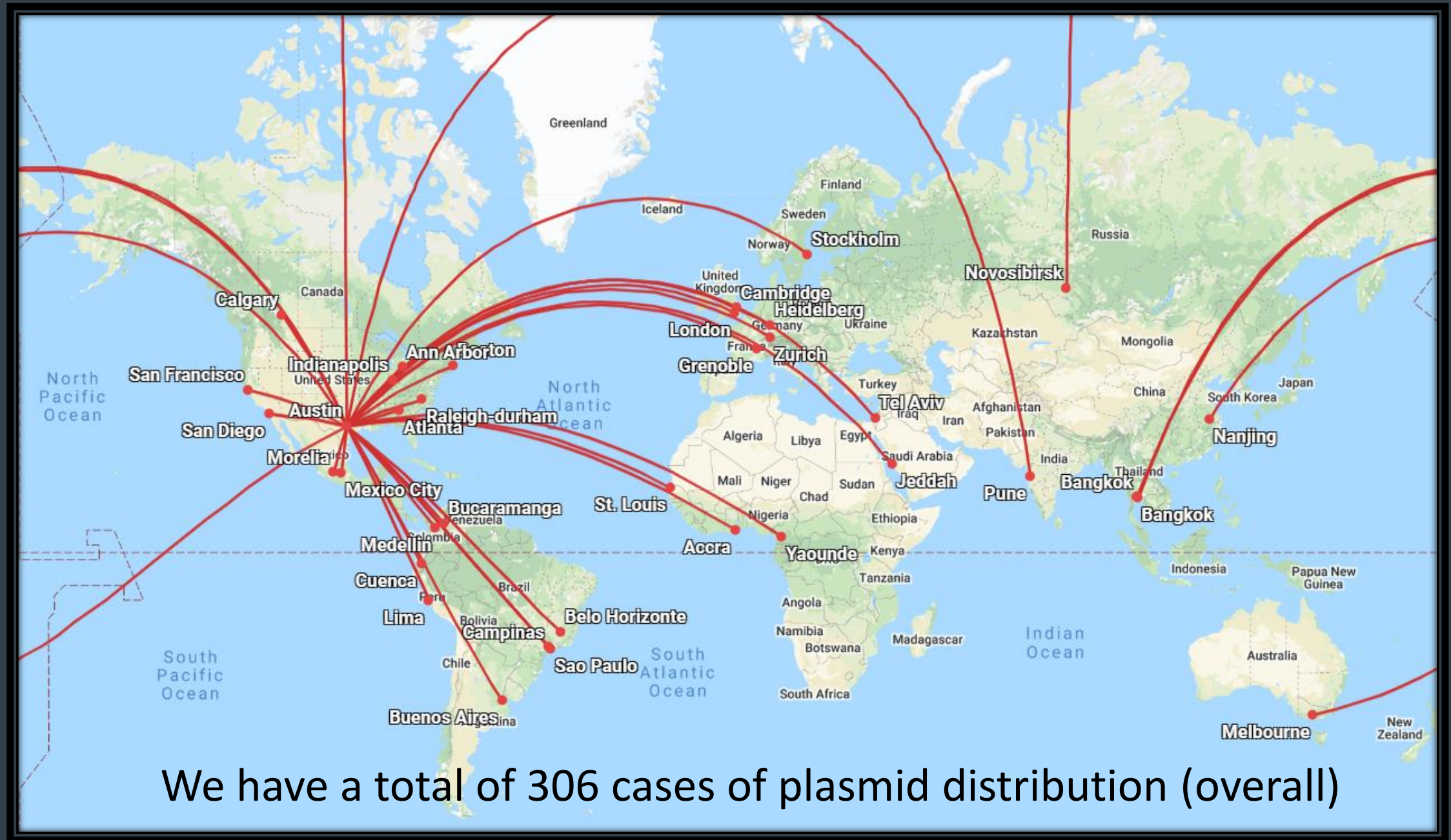
Reagent	Cost per tube ($)	Cost ($) per test (4 tubes)
LAMP Primers	0.11	0.44
OSD probe	0.05	0.08
Bst 2.0	0.57	2.28
RT	1.1	4.4
Betaine	0.04	0.16
dNTP	0.24	0.96
Total	2.1	8.32

- Enzymes are the most expensive ingredient.
- This cost might be reduced by using engineered enzyme alternatives



1. SARS-2 Mx RT-LAMP-OSD no primers (control)
2. SARS-2 Mx RT-LAMP-OSD with primers (test)
3. Human *gapd* no primers (control)
4. Human *gapd* with primers (test)

Ellington Lab's Distribution Efforts (06.01.2020 ~ Current)



We have a total of 306 cases of plasmid distribution (overall)

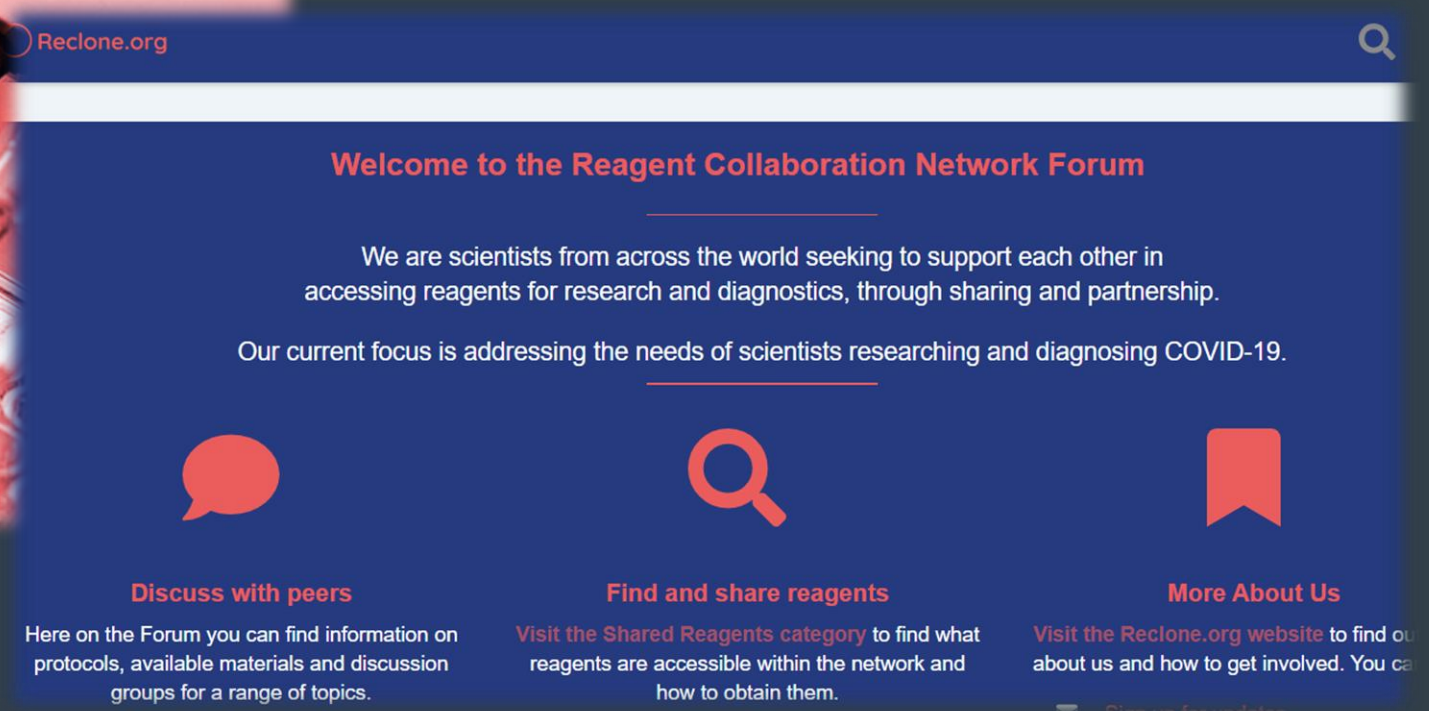
And via our collaborators at Cambridge University!

All thanks to Dr. Jenny Molloy at Cambridge for spearheading these efforts!

Reagents and Protocols, Share and Discussion

Reclone.org

Forum.reclone.org



Acknowledgements

- Just about everything: Sanchita Bhadra
- Enzyme engineering: Andre Maranhao, David Walker, Inyup Paik
- Environmental screening: Dalton Towers, David Brown

Funding: NSF RAPID, NIBIB Supplement, Welch Foundation